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NOTICE OF ALLOWANCE AND FEE(S) DUE

7590 03/04/2004

Reza Green, Esq.
Novo Nordisk of North America, Inc.
Suite 6400
405 Lexington Avenue
New York, NY 10174-6401

EXAMINER	
TRUONG, TAMTHOM NGO	
ART UNIT	**PAPER NUMBER**
1624	

DATE MAILED: 03/04/2004

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/995,137	11/27/2001	Lone Jeppesen	5700.250-US	1962

TITLE OF INVENTION: TRICYCLIC COMPOUNDS AS NUCLEAR RECEPTOR MODULATORS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1330	$300	$1630	06/04/2004

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE REFLECTS A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE APPLIED IN THIS APPLICATION. THE PTOL-85B (OR AN EQUIVALENT) MUST BE RETURNED WITHIN THIS PERIOD EVEN IF NO FEE IS DUE OR THE APPLICATION WILL BE REGARDED AS ABANDONED.

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☐ Applicant claims SMALL ENTITY status. See 37 CFR 1.27.

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(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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nonprovisional	NO	$1330	$300	$1630	06/04/2004

EXAMINER	ART UNIT	CLASS-SUBCLASS
TRUONG, TAMTHOM NGO	1624	514-211030

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

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This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, Alexandria, Virginia 22313-1450.

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7590 03/04/2004

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EXAMINER	
TRUONG, TAMTHOM NGO	
ART UNIT	**PAPER NUMBER**
1624	

DATE MAILED: 03/04/2004

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 0 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 0 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) system (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (703) 305-1383. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at (703) 305-8283.

PTOL-85 (Rev. 11/03) Approved for use through 04/30/2004.

Notice of Allowability	Application No. 09/995,137	Applicant(s) JEPPESEN ET AL.	
	Examiner Tamthom N. Truong	Art Unit 1624	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *the amendment of 12-18-03*.
2. ☒ The allowed claim(s) is/are *1,2,7,16,18,23,24,26-33,36,43,44 and 50-55*.
3. ☐ The drawings filed on _____ are accepted by the Examiner.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.
6. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
   1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
7. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☒ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☒ Information Disclosure Statements (PTO-1449 or PTO/SB/08), Paper No./Mail Date #2
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application (PTO-152)
6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
7. ☐ Examiner's Amendment/Comment
8. ☐ Examiner's Statement of Reasons for Allowance
9. ☒ Other *"Allowable Subject Matter"*.

Allowable Subject Matter

Applicant's amendment of 12-18-03 has overcome the previous rejections of 112/1st paragraph regarding new matter, and the objection to the specification. The specification and claim 1 have been amended to include the original proviso. No new matter is noted. Therefore, the previous rejection and objection are withdrawn herein for the allowance of pending claims 1, 2, 7, 16, 18, 23, 24, 26-33, 36, 43, 44, and 50-55.

The closest reference, **Lohray et. al.** (US 6,054,453), teaches compounds of a tricyclic system in which the ring on the right can be a heterocycle. However, said compounds do not have a middle ring of a seven- or eight-membered containing -N-S(O)$_2$- or –O-CH$_2$-O-. Other references of record all teach a dibenzo-fused tricyclic system, which has been excluded by the proviso of claim 1.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Tamthom N. Truong whose telephone number is 571-272-0676. The examiner can normally be reached on M-T (~10 am ~ 8:30 pm) starting from February 22nd, 2004.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mukund Shah can be reached at 571-272-0674. If you are unable to reach Dr. Shah within a 24 hour period, please contact James O. Wilson, Acting SPE of 1624, at 571-272-0661. The fax phone numbers for the organization where this application or proceeding is assigned are 703-872-9306.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-1235.

T. Truong
***
February 25, 2004

RICHARD L. RAYMOND
ACTING SPE
ART UNIT 1624

#2

Sheet 1 of 1

FORM PTO-1449 (Rev. 2-32)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	Atty. Docket No. 5700.250-US	Serial No. ~~To be assigned~~ 9/995,137
INFORMATION DISCLOSURE STATEMENT BY APPLICANT		Applicant Jeppesen et al.	
(Use several sheets if necessary)		Filing Date November 27, 2001	Group ~~To be assigned~~ 1624

JC979 U.S. PTO 09/995137

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
TNT		6,054,453	04/25/00	Lohray et al.	514	226.2	01/23/98

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
TNT	✓	WO 97/36579	10/09/97	WIPO				
	✓	WO 97/25042	07/17/97	WIPO				
	✓	WO 96/04261	02/15/96	WIPO				
	✓	WO 96/04260	02/15/96	WIPO				
TNT	✓	WO 99/19313	04/22/99	WIPO				

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)

			Abstract of Japanese Patent No. JP 10182550

EXAMINER	[signature]	DATE CONSIDERED	5/9/02

EXAMINER: Initial if citation considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Notice of References Cited	Application/Control No. 09/995,137	Applicant(s)/Patent Under Reexamination JEPPESEN ET AL.	
	Examiner Tamthom N. Truong	Art Unit 1624	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
✓	A	US-5,011,833	04-1991	Barsini et al.	514/211.08
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
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	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
/	U	GIANNOTTI, D. et. al., J. Med. Chem., 1991, Vol. 34, No. 4, pp. 1356-62.
✓	V	SAUERBERG P. et. al., J. Med. Chem., 2002, Vol. 45, No. 4, pp. 789-804.
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

2/28/04

 J. Med. Chem. 1991, 34, 1356–1362

New Dibenzothiadiazepine Derivatives with Antidepressant Activities

Danilo Giannotti,*[†] Giovanni Viti,[†] Piero Sbraci,[†] Vittorio Pestellini,[†] Giovanna Volterra,[‡] Franco Borsini,[‡] Alessandro Lecci,[‡] Alberto Meli,[‡] Paolo Dapporto,[§] and Paola Paoli[§]

Dipartimento Ricerche Chimiche e Farmacologiche, A. Menarini Industrie Farmaceutiche Riunite S.r.l., V. Sette Santi 3, 50131 Firenze, Italy, and Dipartimento di Energetica, Università di Firenze, V.S. Marta 3, 50139 Firenze, Italy.
Received October 11, 1989

A new series of 11-[(aminoalkyl)carbonyl] derivatives of 6,11-dihydrodibenzo[*c,f*][1,2,5]thiadiazepine 5,5-dioxide (10–39) were synthesized and evaluated for potential antidepressant activity in the apomorphine-induced hypothermia (Apo 16) test. Effects on reserpine-induced hypothermia and toxicity for the most potent antagonists of Apo 16 hypothermia were also studied. Structure–activity relationships are reported. Anticholinergic effects were evaluated for compound 12, identified as the most potent and least toxic in this series, by assessing physostigmine lethality. Compound 12 was also subjected to X-ray analysis.

Introduction

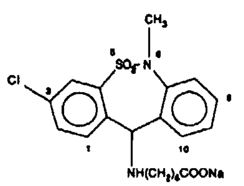
Many antidepressant agents used in clinical practice have a tricyclic structure, with two aromatic rings fused to the central heptatomic ring. An aminoalkyl side chain is attached to the central ring on which one or more heteroatoms can be present.

This paper describes tricyclic compounds where three heteroatoms, a sulfonamido group, and a nitrogen atom are present in the heptatomic ring as in the dibenzothiadiazepines shown in Schemes I and II. Some derivatives of dibenzothiadiazepines with an aminoalkyl chain in position 11 have been reported.[1,2] These compounds were shown to have imipramine-like activity in animal models of depression but were not developed due to adverse reactions on the cardiovascular system.[3]

With the aim of finding a potential antidepressant with fewer side effects than the classical tricyclic antidepressants,[4] we synthesized new dibenzothiadiazepines substituted in position 11 with an (aminoalkyl)carbonyl chain;[5] the introduction of a carbonyl linked to nitrogen in position 11 transforms this basic nitrogen to an amide and therefore may change the pharmacological and toxicological properties of the compounds.

It is known that in imipramine and desipramine, an analogous substitution of the aminoalkyl chain with an (aminoalkyl)carbonyl chain gives rise to products with antidepressant activity;[6,7] in other cases with the same substitution no pharmacological activity was found.[8] No information is given on the effects on toxicity for this substitution, however.

It is noteworthy that tianeptine (example I), a new atypical antidepressant which also has an heptatomic ring containing the sulfonamido group in position 5–6, has few side effects and is active as an antidepressant in clinical trials.[9-11]



tianeptine

Chemistry

The 6,11-dihydrodibenzo[*c,f*][1,2,5]thiadiazepine 5,5-dioxide 7a–l intermediates[12] listed in Table I were prepared by the general procedures outlined in Scheme I.

The reaction of the 2-nitrobenzenesulfonyl chlorides 1 with 2-haloanilines 2 led to the *N*-(2′-halophenyl)-2-nitrobenzenesulfonamides 3a–i; subsequent reduction with boiling Fe/AcOH and acetylation in situ with Ac_2O gives *N*-(2-halophenyl)-2-(acetylamino)benzenesulfonamides 5a–j. Alternatively, these compounds could be directly obtained by treatment of the 2-aminobenzenesulfonyl chlorides 1a with 2 followed by acetylation with Ac_2O. Compounds 5a–j were N-alkylated to *N*-(2′-halophenyl)-*N*-alkyl-2-(acetylamino)benzenesulfonamides 6a–l and cyclized, according to the method of Goldberg,[13] to products 7a–l.

The 11-[(aminoalkyl)carbonyl] derivatives of 6,11-dihydrodibenzo[*c,f*][1,2,5]thiadiazepine 5,5-dioxide 10–39[5] were synthesized as outlined in Scheme II and are summarized in Table II. Refluxing compounds 7a–l with excess of ω-chloroalkanoyl chlorides gave derivatives 9a–m for $n = 1$ or 2 (listed in Table I). Treatment of these derivatives with amines afforded the desired products 12–38.

Compound 39 was obtained by catalytic reduction of the azido derivative obtained from compound 9a.

Products 10 and 11 were synthesized by reaction of the sodium salt of 7a with trichloromethyl chloroformate and subsequent treatment of the intermediate 8a with ammonia to form 10 or with dimethylamine to form 11.

Results and Discussion

Data relative to the antagonism of apomorphine (16 mg/kg) induced hypothermia by the new compounds (400 mg/kg os) are presented in Table II.

The data indicate that the amino group in the (amino-

[†] Dipartimento Ricerche Chimiche, A. Menarini Industrie Farmaceutiche.

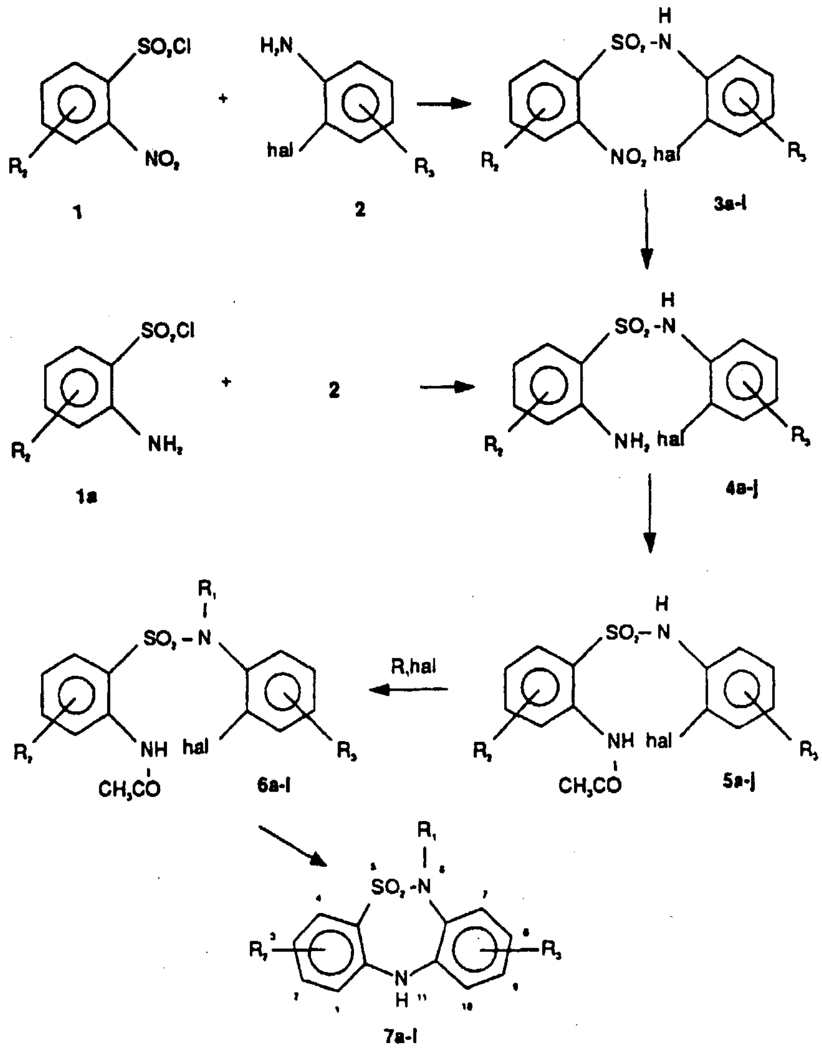
[‡] Dipartimento Ricerche Farmacologiche, A. Menarini Industrie Farmaceutiche.

[§] Dipartimento di Energetica, Università di Firenze.

(1) Weber, A. U.S. Patent 3,274,058, 1966; *Chem. Abstr.* 1967, *66*, 95092.
(2) Kreighbaum, W. E. U.S. Patent 3,322,789, 1967; *Chem. Abstr.* 1967, *67*, 100161.
(3) Weber, A.; Frossard, J. *Ann. Pharm. Fr.* 1966, *24*, 445.
(4) For a review on "second-generation antidepressants", see: Blackwell, B.; Simon, J. S. *Drugs Today* 1986, *22*, 611.
(5) Borsini, F.; Meli, A.; Volterra, G.; Giannotti, D.; Pestellini, V. *Ital. Patent.* 1988, 9466.
(6) Oksenkrug, G. F. *Farmakol. Toksikol.* 1975, *38*, 23.
(7) Oksenkrug, G. F. *J. Pharm. Pharmacol.* 1973, *25*, 1013.
(8) Monro, A. M.; Quinton, R. M.; Wrigley, T. I. *J. Med. Chem.* 1963, *6*, 255.
(9) Labrid, C.; Moleyre, J.; Poignant, J. C.; Malen, C.; Mocaer, E.; Kamoun, A. *Clin. Neuropharmacol.* 1988, *11* (*Suppl. 2*), 21.
(10) Mocaer, E.; Rettori, M. C.; Kamoun, A. *Clin. Neuropharmacol.* 1988, *11* (*Suppl. 2*), 32.
(11) Defrance, R.; Marey, C.; Kamoun, A. *Clin. Neuropharmacol.* 1988, *11* (*Suppl. 2*), 74.
(12) Weber, A. U.S. Patent 3,268,557, 1966; *Chem. Abstr.* 1966, *65*, 15406.
(13) (a) Goldberg, I. *Ber. Dtsch. Chem. Ges.* 1906, *39*, 1691. (b) Goldberg, I. *Ber. Dtsch. Chem. Ges.* 1907, *40*, 4541.

0022-2623/91/1834-1356$02.50/0

Scheme I



alkyl)carbonyl chain is important for the antagonism of apomorphine-induced hypothermia. The terminal amino group of the most active compounds can be secondary (29) or tertiary and linked to a short chain alkyl group (12, 21, 23, 29, 33, 34, 36, 38); decrease in activity can be observed for bulkier or branched alkyl groups (17, 24, 25) and in the absence of the alkyl group (39). Compounds 13–16, 19, 20, and 26–28, where the amino group is part of a heterocycle, were inactive. When bulkier alkyl groups are substituted for the methyl group on the sulfonamide nitrogen (position 6) compounds (22, 32) with lower activity are obtained.

Aromatic substitution in the two benzene rings, in compounds with the dimethylamino group in the side chain, gives compounds (33, 34, 36, 38) with comparable activity to the unsubstituted compound (12) or compounds (18, 30, 31, 35, 37) with lower activity.

The ED_{50} for reversal of Apo 16 induced hypothermia and the minimal dose effective in reducing reserpine-induced hypothermia, as well as the LD_{50} for the more active compounds, are shown in Table III. Imipramine and compounds 40 and 41 (dibenzothiadiazepines which have an aminoalkyl chain substituted on position 11 of the central ring)[3] were used for comparison.

With the exception of 33 and 38 most of the compounds selected had similar efficacy in antagonizing apomorphine-induced hypothermia, while compound 12 was the most active in antagonizing reserpine-induced hypothermia. Some activity in the reserpine test was seen with compounds 29 (a derivative with a secondary terminal amino group) and 34 (a derivative with a methyl aromatic substitution in position 9).

Aromatic substitution (33, 34, 36, 38) caused a decrease in the activity in antagonizing reserpine-hypothermia in comparison with compound 12.

Compound 12 shows that the amino group of the (aminoalkyl)carbonyl chain with the best activity is the dimethylamino group, while compound 29 with the monomethylamino group is more toxic and is also less potent than the dimethylamino compounds (12) in reversing reserpine-induced hypothermia. Compound 23 with the diethylamino group has about half the activity of compound 12 in the reversal of Apo 16 induced hypothermia and has reduced potency in reversing reserpine-induced hypothermia even though it seems to be somewhat less toxic.

The length of the side chain for optimal activity in this series seems to be that where the two nitrogen atoms are separated by a chain of two carbon atoms (12); when the chain is of three carbon atoms, activity decreases (21). This fact is in contrast to what is seen with other imipramine-like compounds, where the side chain is usually of three carbon atoms.

In the dibenzothiadiazepines with a two-carbon side chain, the introduction of a carbonyl group linked to a

Scheme II

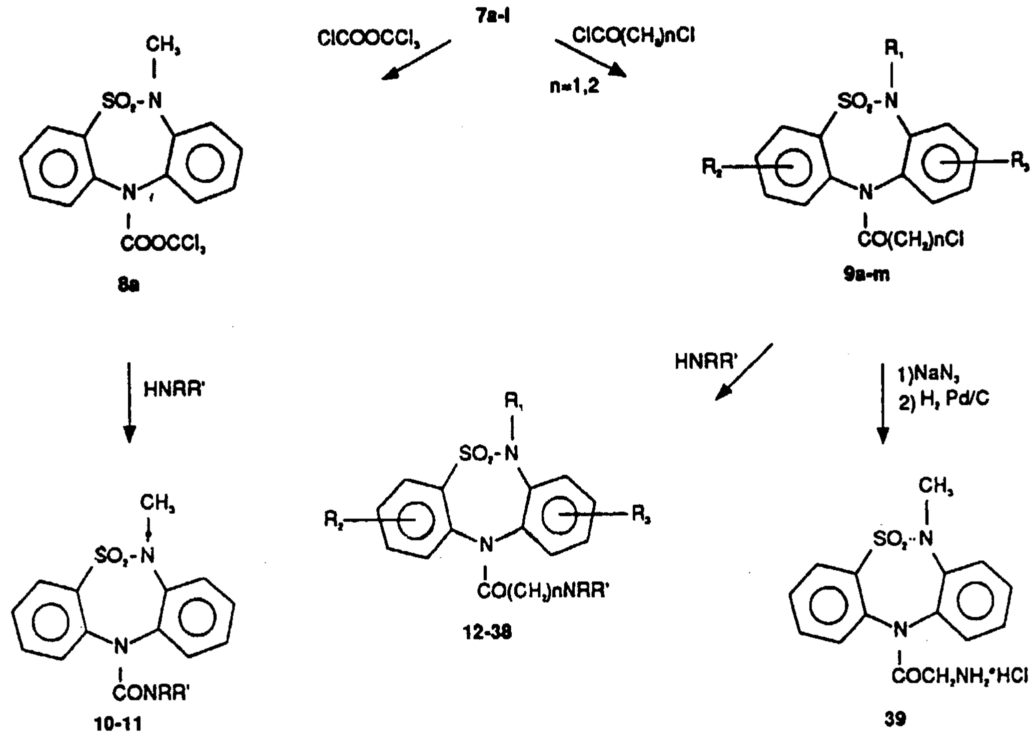
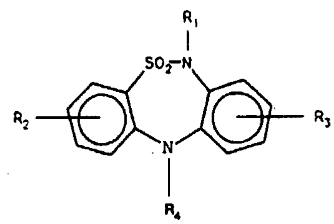


Table I. Physical Properties of Dibenzothiadiazepines 7a–l and 9a–m



no.	R_1	R_2	R_3	R_4	formula[a]	mp, °C (solv)[b]	% yield
7a	CH_3	H	H	H	$C_{13}H_{12}N_2O_2S$	201–202 (E)[c]	91
7b	CH_3	2-Cl	H	H	$C_{13}H_{11}ClN_2O_2S$	308–310 (P)	78
7c	CH_3	2-OCH_3	H	H	$C_{14}H_{14}N_2O_3S$	187–189 (E)	58
7d	CH_3	2-CF_3	H	H	$C_{14}H_{11}F_3N_2O_2S$	231–232 (E)	65
7e	CH_3	H	9-Cl	H	$C_{13}H_{11}ClN_2O_2S$	265 dec. (E)	55
7f	CH_3	H	9-CH_3	H	$C_{14}H_{14}N_2O_2S$	259–260 (A)	62
7g	CH_3	H	8-Cl	H	$C_{13}H_{11}ClN_2O_2S$	225–226 (E)	68
7h	CH_3	2-CH_3	H	H	$C_{14}H_{14}N_2O_2S$	167–169 (P)	88
7i	CH_3	3-Cl	H	H	$C_{13}H_{11}ClN_2O_2S$	267–269 (P)	63
7j	CH_3	2-Cl, 3-CH_3	H	H	$C_{14}H_{13}ClN_2O_2S$	243–244 (E)	72
7k	C_2H_5	H	H	H	$C_{14}H_{14}N_2O_2S$	188–189 (E)	60
7l	C_3H_7	H	H	H	$C_{15}H_{16}N_2O_2S$	186–187 (E)	60
9a	CH_3	H	H	$COCH_2Cl$	$C_{15}H_{13}ClN_2O_3S$	141–143 (L)	80
9b	CH_3	2-Cl	H	$COCH_2Cl$	$C_{15}H_{12}Cl_2N_2O_3S$	183–184 (L)	85
9c	CH_3	2-OCH_3	H	$COCH_2Cl$	$C_{16}H_{15}ClN_2O_4S$	178–179 (E)	73
9d	CH_3	2-CF_3	H	$COCH_2Cl$	$C_{16}H_{12}ClF_3N_2O_3S$	121–122 (E)	76
9e	CH_3	H	9-Cl	$COCH_2Cl$	$C_{15}H_{12}Cl_2N_2O_3S$	202–203 (A)	70
9f	CH_3	H	9-CH_3	$COCH_2Cl$	$C_{16}H_{15}ClN_2O_3S$	155–157 (A)	80
9g	CH_3	H	8-Cl	$COCH_2Cl$	$C_{15}H_{12}Cl_2N_2O_3S$	158–159 (L)	86
9h	CH_3	2-CH_3	H	$COCH_2Cl$	$C_{16}H_{15}ClN_2O_3S$	167–168 (A)	72
9i	CH_3	3-Cl	H	$COCH_2Cl$	$C_{15}H_{12}Cl_2N_2O_3S$	209–210 (A)	90
9j	CH_3	2-Cl, 3-CH_3	H	$COCH_2Cl$	$C_{16}H_{14}Cl_2N_2O_3S$	164–166 (E)	70
9k	C_2H_5	H	H	$COCH_2Cl$	$C_{16}H_{15}ClN_2O_3S$	174–176 (E)	75
9l	C_3H_7	H	H	$COCH_2Cl$	$C_{17}H_{17}ClN_2O_3S$	147–148 (L)	72
9m	CH_3	H	H	$COCH_2CH_2Cl$	$C_{16}H_{15}ClN_2O_3S$	131–132 (E)	80

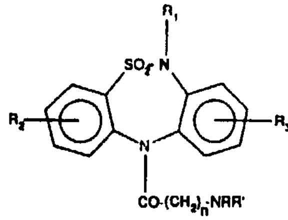
[a] Compounds were analyzed for C, H, and N, and results were within ±0.4% of the theoretical values. [b] Recrystallization solvents: A = acetone, E = ethanol, L = ethyl acetate, P = 1-propanol. [c] Reference 3.

nitrogen in position 11 in the side chain increases overall activity in reversing apomorphine and reserpine hypothermia, and above all brings about a reduction in toxicity.

Finally, compound **12** of this series is the compound with the best therapeutic index, when compared to **40**, **41**, and imipramine.

Further studies on anticholinergic activity, cardiotoxicity, binding, and mechanism of action of this tricyclic

Table II. Physical and Pharmacological Data for 11-(Aminoalkyl)carbonyl Derivatives of Dibenzothiadiazepine



no.	*n*	NRR′	R_1	R_2	R_3	formula[a]	mp, °C (solv)[b]	% yield	apomorphine test: rectal temperature,[c] °C
10	0	NH_2	CH_3	H	H	$C_{14}H_{13}N_3O_3S$	240–242 (A)	60	33.8
11	0	$N(CH_3)_2$	CH_3	H	H	$C_{16}H_{17}N_3O_3S$	170–171 (E)	50	33.7
12	1	$N(CH_3)_2$	CH_3	H	H	$C_{17}H_{19}N_3O_3S$	179–181 (E)	70	38.7*
13	1	4-methylpiperazin-1-yl	CH_3	2-Cl,3-CH_3	H	$C_{21}H_{25}ClN_4O_3S$	156–158 (I)	73	32.5
14	1	4-phenylpiperazin-1-yl	CH_3	2-Cl,3-CH_3	H	$C_{26}H_{27}ClN_4O_3S$	194–196 (A)	66	34.5
15	1	4-(2-hydroxyethyl)piperazin-1-yl	CH_3	2-Cl,3-CH_3	H	$C_{22}H_{27}ClN_4O_4S{\cdot}2HCl$	234–236 (E)	63	34.1
16	1	4-(2-pyrimidyl)piperazin-1-yl	CH_3	2-Cl,3-CH_3	H	$C_{24}H_{25}ClN_6O_3S$	180–182 (T/R)	60	32.7
17	1	$NHCH(CH_3)_2$	CH_3	2-Cl,3-CH_3	H	$C_{19}H_{22}ClN_3O_3S{\cdot}HCl$	248–250 (A)	50	33.1
18	1	$N(CH_3)_2$	CH_3	2-Cl,3-CH_3	H	$C_{18}H_{20}ClN_3O_3S{\cdot}HCl$	240–242 (E)	80	35.8
19	1	4-methylpiperazin-1-yl	CH_3	H	H	$C_{20}H_{24}N_4O_3S$	143–144 (I)	70	34.4
20	1	4-(2-hydroxyethyl)piperazin-1-yl	CH_3	H	H	$C_{21}H_{26}N_4O_4S{\cdot}2HCl$	236–238 (E)	60	34.4
21	2	$N(CH_3)_2$	CH_3	H	H	$C_{18}H_{21}N_3O_3S{\cdot}HCl$	189–190 (A)	68	36.5*
22	1	$N(CH_3)_2$	C_2H_5	H	H	$C_{18}H_{21}N_3O_3S$	115–116 (C)	20	35.1
23	1	$N(C_2H_5)_2$	CH_3	H	H	$C_{19}H_{23}N_3O_3S$	161–162 (E)	67	37.4*
24	1	$NHCH(CH_3)_2$	CH_3	H	H	$C_{18}H_{21}N_3O_3S$	115–117 (E)	60	34.1
25	1	$NHC(CH_3)_3$	CH_3	H	H	$C_{19}H_{23}N_3O_3S$	143–144 (E)	64	32.2
26	1	morpholin-1-yl	CH_3	H	H	$C_{19}H_{21}N_3O_4S$	129–131 (C)	70	33.9
27	1	4-methylpiperidin-1-yl	CH_3	H	H	$C_{21}H_{25}N_3O_3S$	159–160 (E)	61	33.2
28	1	pyrrolidin-1-yl	CH_3	H	H	$C_{19}H_{21}N_3O_3S$	175–177 (E)	67	34.2
29	1	$NHCH_3$	CH_3	H	H	$C_{16}H_{17}N_3O_3S$	135–137 (L)	45	38.2*
30	1	$N(CH_3)_2$	CH_3	2-Cl	H	$C_{17}H_{18}ClN_3O_3S$	138–139 (I)	75	35.4
31	1	$N(CH_3)_2$	CH_3	2-OCH_3	H	$C_{18}H_{21}N_3O_4S{\cdot}HCl$	270–272 (E)	70	33.6
32	1	$N(CH_3)_2$	C_3H_7	H	H	$C_{19}H_{23}N_3O_3S{\cdot}HCl$	147–149 (I)	77	35.8
33	1	$N(CH_3)_2$	CH_3	H	9-Cl	$C_{17}H_{18}ClN_3O_3S$	175–176 (P)	70	37.4*
34	1	$N(CH_3)_2$	CH_3	H	9-CH_3	$C_{18}H_{21}N_3O_3S$	135–137 (E)	78	38.8*
35	1	$N(CH_3)_2$	CH_3	2-CF_3	H	$C_{18}H_{18}F_3N_3O_3S$	156–157 (I)	63	34.8
36	1	$N(CH_3)_2$	CH_3	H	8-Cl	$C_{17}H_{18}ClN_3O_3S$	132–133 (I)	82	39.5*
37	1	$N(CH_3)_2$	CH_3	2-CH_3	H	$C_{18}H_{21}N_3O_3S$	159–160 (I)	74	35.2
38	1	$N(CH_3)_2$	CH_3	3-Cl	H	$C_{17}H_{18}ClN_3O_3S{\cdot}HCl$	163–165 dec (I)	65	37.7*
39	1	NH_2	CH_3	H	H	$C_{15}H_{15}N_3O_3S{\cdot}HCl$	242–244 (I)	46	36.1

[a] Compounds were analyzed for C, H, and N, and results were within ±0.4% of the theoretical values. [b] Recrystallization solvents: A = acetone, C = cyclohexane, E = ethanol, I = 2-propanol, L = ethyl acetate, T = tetrahydrofuran, R = ethyl ether, P = 1-propanol. [c] Values of rectal temperature represent the mean from 8–10 mice. The standard errors (omitted) were in the range of 1–3% of the mean. Compounds were administered orally at a dose of 400 mg/kg 0.5 h before the rectal temperature measurement. Apomorphine (16 mg/kg) was injected subcutaneously 30 min before the rectal temperature recording. The rectal temperature of vehicle- and apomorphine-treated mice ranged from 36.7 to 37.2 and from 33 to 34 °C, respectively. * $P < 0.01$ from rectal temperature of apomorphine-treated mice.

with potential antidepressant activity were therefore carried out.

Since anticholinergic side-effects are known to be very common with tricyclic antidepressants,[14] the effect of compound 12, imipramine, and amitriptyline on physostigmine lethality were compared (Table IV). Results clearly indicate that compound 12 has far less anticholinergic effects than imipramine and amitriptyline. Tricyclic antidepressants have direct myocardial effects due to high affinity for cardiac tissue. Cardiac activity is reflected by electrocardiographic changes and changes in contractile activity.[14] Compound 12 was compared to imipramine in its effects on heart rate both in vivo and in vitro and on the *R*/*S* ratio in the ECG. It had far less cardiotoxicity than imipramine.[15]

It is interesting that as far as binding studies for compound 12 are concerned, binding was not found to any of the various receptors examined (α- and β-adrenergic, do-

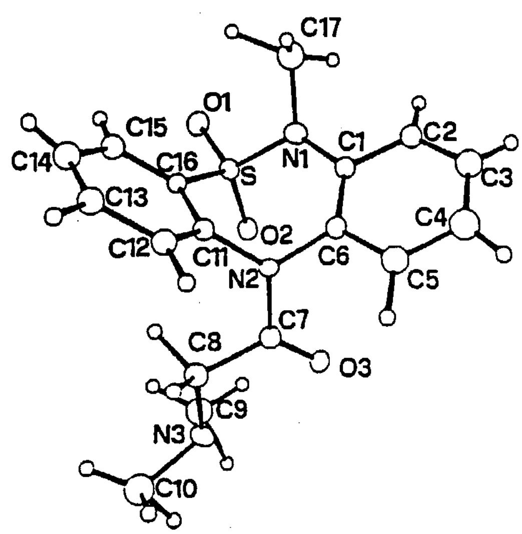
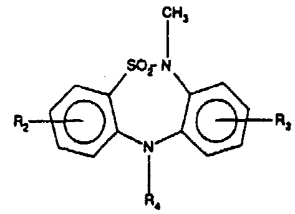


Figure 1. ORTEP drawing of 12.

(14) Baldessarini, R. J. In *The Pharmacological Basis of Therapeutic*, 7th ed.; Gilman, A. G., Goodman, L. S., Rall, T. W., Murad, F., Eds.; MacMillan Publishing Co.: New York, 1985; Chapter 19.

(15) Borsini, F.; Volterra, G.; Lecci, A.; Evangelista, S.; Mancinelli, A.; Cutrufo, C.; Parlani, M.; Mennini, T.; Barone, D.; Meli, A. *Arzneim.-Forsch.*, in press.

Table III. Pharmacological Results for Representative 11-[(Aminoalkyl)carbonyl] Derivatives of Dibenzothiadiazepine



no.	R_4	R_2	R_3	hypothermia inhibition: ED_{50} Apo 16[a]	hypothermia inhibition: minimal effective dose: reserpine test[b]	LD_{50}[c]
12	$COCH_2N(CH_3)_2$	H	H	13.5 (4.1-22.6)	25	1920 (1802-2064)
21	$COCH_2CH_2N(CH_3)_2$	H	H	23.1 (11.3-94.9)	>100	521 (374-726)
23	$COCH_2N(C_2H_5)_2$	H	H	23 (3.4-71.1)	>100	>2000[d]
29	$COCH_2NHCH_3$	H	H	12 (1.2-22.2)	100	789 (613-1015)
33	$COCH_2N(CH_3)_2$	H	9-Cl	50.6 (34-98.6)	>100	NT[e]
34	$COCH_2N(CH_3)_2$	H	9-CH_3	14.4 (10.2-24.7)	100	1734 (1525-1972)
36	$COCH_2N(CH_3)_2$	H	8-Cl	23.5 (15.3-43.9)	>100	1424 (1136-1785)
38	$COCH_2N(CH_3)_2$	3-Cl	H	54.5 (26.1-102.2)	>100	NT
40	$CH_2CH_2N(CH_3)_2$	H	H	>100	100	468 (387-564)
41	$CH_2CH_2CH_2N(CH_3)_2$	H	H	4.9 (1.3-11.1)	>100	655 (542-792)
	imipramine			3.7 (1.1-4.8)	4	343 (263-448)

[a] See legend of Table II for apomorphine test. ED_{50}: dose of compound reducing apomorphine-induced hypothermia by 50%; 95% confidence limits are reported in brackets. [b] Reserpine (2.5 mg/kg) was administered subcutaneously 18 h before the rectal-temperature recording. Compounds were given orally at time of peak activity in general 0.5 h before rectal-temperature recording. [c] LD_{50}: dose of compound in mg/kg inducing lethality in 50% of mice after 14 days; 95% confidence limits are reported in parentheses. [d] 30% at 2 g/kg. [e] NT: not tested.

Table IV. Effect of Compound 12, Imipramine, and Amitriptyline on Physostigmine-Induced Lethality

treatment	dose, mg/kg	physostigmine, % of lethality[a]
vehicle		100
compound 12	200	95
compound 12	400	95
compound 12	600	90
amitriptyline	25	37*
imipramine	50	67*
imipramine	25	95

[a] Compounds were orally administered 60 min before physostigmine (0.9 mg/kg) intraperitoneal injection. Lethality was evaluated 24 h later. * $P < 0.01$ (χ^2) vs vehicle.

pamine, serotonin, histamine, benzodiazepine, GABA, muscarinic, imipramine, and desipramine).[15] Similar results were found for tianeptine.[16]

Unlike imipramine, compound 12 does not inhibit 5HT or NA uptake,[15] on the contrary, like tianeptine,[17] compound 12 enhances 5HT uptake.[15]

In addition to the above, conformational studies were carried out for compound 12; in Figure 1 an ORTEP view of the structure as determined by X-ray analysis is shown.

There are some structural characteristics that are analogous to those of the classical tricyclic antidepressants: (1) The dihedral angle between the two benzene rings is 124°, this is important as the average folding angle is about 120° in antidepressants while it is 155° in neuroleptics.[18]

(2) The (aminoalkyl)carbonyl chain lies in the opposite direction to the tricyclic arrangement and displays a conformation close to the trans one: the dihedral angle N2-C7-C8-N3 is 170.7 (9)°. This arrangement of the side chain is found in X-ray analysis of imipramine and in other tricyclic antidepressants.[19,20]

(3) The distance of the nitrogen N3 of the side chain to the center of the nearest ring is 5.15 Å. A similar distance was found in the correlation analysis of conformations of classical tricyclic antidepressants (5-5.5 Å),[21] whereas a different distance (6-7 Å) was found for the energetically favored conformations of pirenzepine, a tricyclic compound devoid of antidepressant activity.[22]

Compound 12, however, also presents some features which are characteristic of this product: the geometry around the N2 atom shows an sp^2 hybridization for this atom, this feature is indicative of a large conjugation of the double bond in the N2-C7-O3 moiety; the presence of intramolecular hydrogen bonds as C9-H91···O3 (the H91···O3 distance is 2.62 Å), C15-H15···O1 (the H15···O1 distance is 2.60 Å), and C17-H171···O1 (the H171···O1 distance is 2.22 Å) stabilize the molecular conformation. Some intermolecular hydrogen bonds that involve the O2 and the O3 oxygen atoms are present. This conformational study of compound 12, shows that there are important structural similarities with classical antidepressant tricyclics. Compound 12, and other tricyclic antidepressants also have the same profile of activity in the apomorphine test.[23]

In spite of these structural and pharmacological analogies, the fact that compound 12, unlike other tricyclic antidepressants, does not inhibit monoamine uptake could indicate that if a common mechanism exists, some other biochemical property, shared by tricyclic antidepressants, could be important for antidepressant activity.

In any case further conformational and structural studies are necessary to try to better elucidate similarities and

(16) Kato, G.; Weitsch, A. F. *Clin. Neuropharmacol.* 1988, *11*, S43.
(17) Mennini, T.; Mocaer, E.; Garattini, S. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1987, *336*, 478.
(18) Pepe, G.; Reboul, J. P.; Cristan, B. *Eur. J. Med. Chem.* 1989, *24*, 1.
(19) Tollenaere, J. P.; Moereels, H.; Raymaekers, L. A. *Atlas Of The Three-Dimensional Structure Of Drugs*; Elsevier: North-Holland, 1979; Vol. 1, Chapter 2.
(20) Andrews, P. R.; Lloyd, E. J. *Med. Res. Rev.* 1982, *2*, 355.
(21) De Paulis, T.; Kelder, D.; Ross, S. B. *Mol. Pharmacol.* 1978, *14*, 596.
(22) Trummlitz, G.; Schmidt, G.; Wagner, H. U.; Luger, P. *Arzneim.-Forsch.* 1984, *34*, 849.
(23) Puech, A. J.; Chermat, R.; Poncelet, M.; Doaré, L.; Simon, P. *Psychopharmacology* 1981, *75*, 84.

differences between our compound 12, tianeptine, and other antidepressants.

Experimental Procedure

Pharmacology. Male Swiss albino mice (Nossan, Milan, Italy), 20–22 g, were used. The potential antidepressant activity of compounds was evaluated by the apomorphine[23] and reserpine tests.[24] A preliminary "screening" on apomorphine 16 mg/kg sc (Apo 16) induced hypothermia with a high dose (400 mg/kg os) of the various compounds with temperature measurement at 0.5, 2, 4 h after administration of the product was carried out (Table II). Results are expressed for time of peak activity which was generally 0.5 h.

On subsequent evaluation, the efficacy of active compounds was quantified as the dose reducing the hypothermic response of apomorphine (ED_{50}) by 50% at time of peak activity (0.5–2 h). When the reserpine test was used, the minimal dose of compound capable of reducing significantly reserpine-induced hypothermia was determined.

In studies on physostigmine lethality, physostigmine was administered as a 0.9 mg/kg ip dose 1 h after oral administration of the compounds. Lethal effects were evaluated 24 h after this. The 50% lethal dose of compounds was evaluated for up to 2 weeks from their administration.

In all cases, each experimental group consisted of 8–20 mice.

Chemistry. Analyses indicated by the symbols of the elements were within ±0.4% of the theoretical value and were conducted by the Analytical Department of Menarini. Melting points were determined on a Büchi apparatus in open capillaries and are uncorrected. ^1H NMR spectra were recorded on a Varian EM-360L spectrometer with tetramethylsilane (TMS) as internal standard. IR spectra were recorded on a Perkin-Elmer FT 1710 spectrophotometer. Arylsulfonyl chlorides 1 and 1a were obtained according to known methods;[25,26] 2-haloanilines 2 are commercially available products, only 2-bromo-5-chloroaniline was synthesized as reported.[27]

General Procedure for the Preparation of *N*-(2-Halophenyl)-2-nitrobenzenesulfonamides (3a–j). Substituted 2-nitrobenzenesulfonyl chlorides 1 (0.1 mol) in tetrahydrofuran (80 mL) was added dropwise to a solution of substituted 2-haloanilines 2 (0.1 mol) in pyridine (0.15 mol) and refluxed for 2 h. After this time, solvent was removed, and the residue was washed with 10% HCl and with water, dried, and crystallized to give the following.

N-(2-bromophenyl)-2-nitrobenzenesulfonamide (**3a**; R_2 = H, R_3 = H) (lit.[3] m. p. 147 °C), *N*-(2-bromophenyl)-2-nitro-4-chlorobenzenesulfonamide (**3b**; R_2 = 4-Cl, R_3 = H) [60%, mp (EtOH) 143–144 °C. Anal. ($C_{12}H_8BrClN_2O_4S$) C, H, N.], *N*-(2-bromophenyl)-2-nitro-4-methoxybenzenesulfonamide (**3c**; R_2 = 4-OCH_3, R_3 = H) [55%, mp (EtOH) 95–97 °C. Anal. ($C_{13}H_{11}BrN_2O_5S$) C, H, N.], *N*-(2-bromophenyl)-2-nitro-4-(trifluoromethyl)benzenesulfonamide (**3d**; R_2 = 4-CF_3, R_3 = H) [48%, mp (EtOH) 130–131 °C. Anal. ($C_{13}H_8BrF_3N_2O_4S$) C, H, N.], *N*-(2,4-dichlorophenyl)-2-nitrobenzenesulfonamide (**3e**; R_2 = H, R_3 = 4-Cl) [58%, mp (EtOH) 121–122 °C. Anal. ($C_{12}H_8Cl_2N_2O_4S$) C, H, N.], *N*-(4-methyl-2-bromophenyl)-2-nitrobenzenesulfonamide (**3f**; R_2 = H, R_3 = 4-CH_3) [64%, mp (EtOH) 138–139 °C. Anal. ($C_{13}H_{11}BrN_2O_4S$) C, H, N.], *N*-(5-chloro-2-bromophenyl)-2-nitrobenzenesulfonamide (**3g**; R_2 = H, R_3 = 5-Cl) [74%, mp (EtOH) 128–129 °C. Anal. ($C_{12}H_8BrClN_2O_4S$) C, H, N.], *N*-(2-bromophenyl)-4-methyl-2-nitrobenzenesulfonamide (**3h**; R_2 = 4-CH_3, R_3 = H) [71%, mp (EtOH) 137–139 °C. Anal. ($C_{13}H_{11}BrN_2O_4S$) C, H, N.], and *N*-(2-bromophenyl)-2-nitro-5-chlorobenzenesulfonamide (**3i**; R_2 = 5-Cl, R_3 = H) [85%, mp (EtOH) 144–145 °C. Anal. ($C_{12}H_8BrClN_2O_4S$) C, H, N.].

Synthesis of *N*-(2-Bromophenyl)-4-chloro-5-methyl-2-amidobenzenesulfonamide (4j). 2-Amino-4-chloro-5-methylbenzenesulfonyl chloride (24 g, 0.1 mol) in tetrahydrofuran (80 mL) was added dropwise to a solution of 2-bromoaniline (17.2 g, 0.1 mol) in pyridine (15 mL) and refluxed for 2 h. After this time, solvent was removed, and the solid residue was washed with 10% HCl and with water, dried, and crystallized from EtOH to yield 30 g (80%) of **4j**, mp 158–159 °C. Anal. ($C_{13}H_{12}BrClN_2O_2S$) C, H, N.

General Procedure for the Preparation of *N*-(2-Halophenyl)-2-(acetylamino)benzenesulfonamides 5a–i. Hydrogen-reduced iron powder (0.5 mol) was added portionwise to a refluxing solution of substituted **3a–i** (0.1 mol) in acetic acid (300 mL). After 1 h of reflux, the hot mixture was filtered by suction. The filtrate was treated with 10 mL of acetic anhydride and was allowed to stand overnight. After dilution with water the precipitate was collected, washed with water, dried, and crystallized to give the following: *N*-(2-bromophenyl)-2-(acetylamino)benzenesulfonamide (**5a**; R_2 = H, R_3 = H) [83%, mp (EtOH) 170–171 °C. Anal. ($C_{14}H_{13}BrN_2O_3S$) C, H, N.], *N*-(2-bromophenyl)-2-(acetylamino)-4-chlorobenzenesulfonamide (**5b**; R_2 = 4-Cl, R_3 = H) [88%, mp (EtOH) 145–147 °C. Anal. ($C_{14}H_{12}BrClN_2O_3S$) C, H, N.], *N*-(2-bromophenyl)-2-(acetylamino)-4-methoxybenzenesulfonamide (**5c**; R_2 = 4-OCH_3, R_3 = H) [90%, mp (EtOH) 129–130 °C. Anal. ($C_{15}H_{15}BrN_2O_4S$) C, H, N.], *N*-(2-bromophenyl)-2-(acetylamino)-4-(trifluoromethyl)benzenesulfonamide (**5d**; R_2 = 4-CF_3, R_3 = H) [90%, mp (EtOH) 187–188 °C. Anal. ($C_{15}H_{12}BrF_3N_2O_3S$) C, H, N.], *N*-(2,4-dichlorophenyl)-2-(acetylamino)benzenesulfonamide (**5e**; R_2 = H, R_3 = 4-Cl) [97%, mp (EtOH) 170–171 °C. Anal. ($C_{14}H_{12}Cl_2N_2O_3S$) C, H, N.], *N*-(4-methyl-2-bromophenyl)-2-(acetylamino)benzenesulfonamide (**5f**; R_2 = H, R_3 = 4-CH_3) [95%, mp (EtOH) 157–158 °C. Anal. ($C_{15}H_{15}BrN_2O_3S$) C, H, N.], *N*-(2-bromo-5-chlorophenyl)-2-(acetylamino)benzenesulfonamide (**5g**; R_2 = H, R_3 = 5-Cl), [88%, mp (EtOH) 180–182 °C. Anal. ($C_{14}H_{12}BrClN_2O_3S$) C, H, N.], *N*-(2-bromophenyl)-4-methyl-2-(acetylamino)benzenesulfonamide (**5h**; R_2 = 4-CH_3, R_3 = H) [96%, mp (EtOH) 150–151 °C. Anal. ($C_{15}H_{15}BrN_2O_3S$) C, H, N.], and *N*-(2-bromophenyl)-5-chloro-2-(acetylamino)benzenesulfonamide (**5i**; R_2 = 5-Cl, R_3 = H) [93%, mp (EtOH) 186–188 °C. Anal. ($C_{14}H_{12}BrClN_2O_3S$) C, H, N.].

Synthesis of *N*-(2-Bromophenyl)-4-chloro-5-methyl-2-(acetylamino)benzenesulfonamide (5j). Acetic anhydride (4 mL) was added to a solution of **4j** (15 g, 0.04 mol) in acetic acid (100 mL) and the mixture was kept at room temperature overnight. Dilution with water gave a precipitate that was collected, washed with water, dried, and crystallized from EtOH to yield 15 g (90%) of **5j**, mp 149–150 °C. Anal. ($C_{15}H_{14}BrClN_2O_3S$) C, H, N.

General Procedure for the Preparation of *N*-(2-Halophenyl)-*N*-alkyl-2-(acetylamino)benzenesulfonamides 6a–l. Substituted **5a–j** (0.1 mol) was added to a solution of sodium (0.1 mol) in methyl alcohol (250 mL) with stirring. After dissolving, the appropriate alkyl halides were added with stirring, and the resulting solution either was kept for 24 h at room temperature (**6a–j**) or was refluxed for 4 h (**6k–l**).

The solution was evaporated, the residue was washed with water, dried, and crystallized to give the following: *N*-(2-bromophenyl)-*N*-methyl-2-(acetylamino)benzenesulfonamide (**6a**; R_1 = CH_3, R_2 = H, R_3 = H) [92%, mp (EtOH) 92–93 °C (lit.[3] mp 94 °C).], *N*-(2-bromophenyl)-*N*-methyl-2-(acetylamino)-4-chlorobenzenesulfonamide (**6b**; R_1 = CH_3, R_2 = 4-Cl, R_3 = H) [90%, mp (EtOH) 116–117 °C. Anal. ($C_{15}H_{14}BrClN_2O_3S$) C, H, N.], *N*-(2-bromophenyl)-*N*-methyl-4-methoxy-2-(acetylamino)benzenesulfonamide (**6c**; R_1 = CH_3, R_2 = 4-OCH_3, R_3 = H) [88%, mp (EtOH) 105–106 °C. Anal. ($C_{16}H_{17}BrN_2O_4S$) C, H, N.], *N*-(2-bromophenyl)-*N*-methyl-4-(trifluoromethyl)-2-(acetylamino)benzenesulfonamide (**6d**; R_1 = CH_3, R_2 = 4-CF_3, R_3 = H) [90%, mp (cyclohexane) 91–92 °C. Anal. ($C_{16}H_{14}BrF_3N_2O_3S$) C, H, N.], *N*-(2,4-dichlorophenyl)-*N*-methyl-2-(acetylamino)benzenesulfonamide (**6e**; R_1 = CH_3, R_2 = H, R_3 = 4-Cl) [90%, mp (*i*-PrOH) 90–91 °C. Anal. ($C_{15}H_{14}Cl_2N_2O_3S$) C, H, N.], *N*-(4-methyl-2-bromophenyl)-*N*-methyl-2-(acetylamino)benzenesulfonamide (**6f**; R_1 = CH_3, R_2 = H, R_3 = 4-CH_3) [96%, mp (*i*-PrOH) 93–94 °C. Anal. ($C_{16}H_{17}BrN_2O_3S$) C, H, N.], *N*-(2-bromo-5-chlorophenyl)-*N*-methyl-2-(acetylamino)benzenesulfonamide (**6g**; R_1 = CH_3, R_2 = H, R_3 - 5-Cl) [95%, mp (EtOH) 109–111 °C. Anal. ($C_{15}H_{14}BrClN_2O_3S$) C, H, N.], *N*-(2-bromo-

(24) Bourin, M.; Poncelet, M.; Chermat, R.; Simon, P. *Arzneim.-Forsch/Drug Res.* **1983**, *33*, 1173.
(25) Meerwein, H.; Dittmar, G.; Gollner, R.; Hafner, K.; Mensch, F.; Steinfort, O. *Chem. Ber.* **1957**, *90*, 841.
(26) Bierbaum, B. A.; Traverso, J. J.; Whitehead, C. W. *J. Med. Chem.* **1963**, *6*, 272.
(27) Mahajanshetti, C. S.; Patil, V. D.; Acharya, S. P.; Nargund, K. S. *J. Karnatak Univ.* **1961**, *6*, 9; *Chem. Abstr.* **1963**, *59*, 8636.

phenyl)-*N*-methyl-4-methyl-2-(acetylamino)benzenesulfonamide (**6h**; $R_1 = CH_3$, $R_2 = 4\text{-}CH_3$, $R_3 = H$) [98%, mp (EtOH) 115–117 °C. Anal. ($C_{16}H_{17}BrN_2O_3S$) C, H, N.], *N*-(2-bromophenyl)-*N*-methyl-2-(acetylamino)-5-chlorobenzenesulfonamide (**6i**; $R_1 = CH_3$, $R_2 = 5\text{-}Cl$, $R_3 = H$) [94%, mp (EtOH) 120–121 °C. Anal. ($C_{15}H_{14}BrClN_2O_3S$) C, H, N.], *N*-(2-bromophenyl)-*N*-methyl-4-chloro-5-methyl-2-(acetylamino)benzenesulfonamide (**6j**; $R_1 = CH_3$; $R_2 = 4\text{-}Cl, 5\text{-}CH_3$; $R_3 = H$) [84%, mp (EtOH) 148–149 °C. Anal. ($C_{16}H_{16}BrClN_2SO_3$) C, H, N.], *N*-(2-bromophenyl)-*N*-ethyl-2-(acetylamino)benzenesulfonamide (**6k**; $R_1 = C_2H_5$, $R_2 = H$, $R_3 = H$) [70%, mp (EtOH) 92–93 °C. Anal. ($C_{16}H_{17}BrN_2O_3S$) C, H, N.], and *N*-(2-bromophenyl)-*N*-propyl-2-(acetylamino)-benzenesulfonamide (**6l**; $R_1 = C_3H_7$, $R_2 = H$, $R_3 = H$) [76%, mp (EtOH) 87–88 °C. Anal. ($C_{17}H_{19}BrN_2O_3S$) C, H, N.].

General Procedure for the Preparation of 6,11-Dihydrodibenzo[*c*,*f*][1,2,5]thiadiazepine 5,5-Dioxides 7a–l. A solution of substituted **6a–l** (0.1 mol) in DMF (400 mL) was treated with K_2CO_3 (13.8 g, 0.1 mol), copper powder (3 g), and CuBr (1.5 g), stirred, and heated to reflux for 8 h. After cooling, the reaction mixture was filtered and the solution diluted with water; the precipitate was collected, washed with water, dried, and crystallized to give products as outlined in Table I.

6-Methyl-6,11-dihydro-11-(chloroacetyl)dibenzo[*c*,*f*]-[1,2,5]thiadiazepine 5,5-Dioxide (9a). **7a** (15 g, 0.057 mol) in chloroacetyl chloride (100 mL) was refluxed for 2 h. The cooled mixture was poured on ice and stirred until decomposition of the acid chloride and solidification of the product. The solid was collected, washed with water, dried, and crystallized from ethyl acetate to yield 15.3 g (80%) of **9a**: mp 141–143 °C; IR (Nujol) 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.3 (s, 3 H, CH_3), 4.05 (s, 2 H, CH_2), 7.2–8.2 (m, 8 H, arom). Anal. ($C_{15}H_{13}ClN_2O_3S$) C, H, N.

Similar reactions with the appropriate 6,11-dihydrodibenzo-[*c*,*f*][1,2,5]thiadiazepine 5,5-dioxides gave the products **9b–m** shown in Table I.

6-Methyl-6,11-dihydro-11-[(*N*,*N*-dimethylamino)acetyl]-dibenzo[*c*,*f*][1,2,5]thiadiazepine 5,5-Dioxide (12). A solution of **9a** (7 g, 0.0206 mol) in acetone (70 mL) was treated with dimethylamine (40 wt% solution in water, 7 mL) and stirred at room temperature. After 2 days the reaction mixture was concentrated. The residue was dissolved in HCl 10% and the insoluble part removed. After alkalinization with Na_2CO_3 of the watery solution, the precipitate was collected, washed with water, dried, and crystallized from EtOH to yield 5 g (70%) of **12**: mp 179–181 °C; IR (Nujol) 1689 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.3 (s, 6 H, $N(CH_3)_2$), 3.05 (s, 2 H, CH_2), 3.3 (s, 3 H, CH_3), 7.1–8 (m, 8 H, arom). Anal. ($C_{17}H_{19}N_3O_3S$) C, H, N.

With a similar procedure starting from the appropriate compounds **9a–m** and alkylamines, the products **17**, **18**, **21**, **22**, **24**, **25**, and **29–38** shown in Table II were obtained.

2-Chloro-3,6-dimethyl-6,11-dihydro-11-[(4-methylpiperazin-1-yl)acetyl]dibenzo[*c*,*f*][1,2,5]thiadiazepine 5,5-Dioxide (13). A solution of**9j** (5 g, 0.013 mol) and of *N*-methylpiperazine (3 mL, 0.02 mol) in acetone (50 mL) was refluxed for 2 h. The mixture was then evaporated, the residue was dissolved in HCl 10%, and the insoluble part was removed. The watery solution was alkalinized with Na_2CO_3, the precipitate collected, washed with water, dried, and crystallized from 2-PrOH to yield 4.3 g (74%) of **13**: mp 156–158 °C; IR (Nujol) 1693 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.8–2.4 (m, 14 H, 2 CH_3 + piperaz), 2.8–3.3 (m, 5 H, CH_2 + CH_3), 7.2–7.9 (m, 6 H, arom). Anal. ($C_{21}H_{25}ClN_4O_3S$) C, H, N.

A similar procedure starting from the appropriate compounds **9a–m** and amines gave the products **14–16**, **19**, **20**, **23**, and **26–28** shown in Table II.

6-Methyl-6,11-dihydro-11-carbamoyldibenzo[*c*,*f*][1,2,5]-thiadiazepine 5,5-Dioxide (10). NaH in oil (about 80%, 0.9 g) was added to a stirred solution of **7a** (7 g, 0.027 mol) in dioxane (200 mL) and kept at 80 °C for 30 min. After cooling in a ice-bath, trichloromethyl chloroformate (5.4 g, 0.027 mol) was added and after 1 h the mixture was saturated with gaseous NH_3. The mixture was filtered, the solvent was removed and the residue washed with water, dried, and crystallized from EtOH to yield 4.9 g (60%) of **10**: mp 240–242 °C; IR (Nujol) 1685 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.3 (s, 3 H, CH_3), 6.2 (s, 2 H, NH_2), 7.1–7.8 (m, 8 H, arom). Anal. ($C_{14}H_{13}N_3O_3S$) C, H, N.

Table V. Crystal and Refinement Data for 12

mol formula	$C_{17}H_{19}N_3O_3S$
mol weight	329.42
a, Å	8.829 (3)
b, Å	8.297 (8)
c, Å	23.655 (8)
β, deg	92.74 (3)
V, Å^3	1712.0 (4)
Z	4
space group	$P2_1/c$
d_{calc}, g·cm^{-3}	1.28
radiation	graphite monochromated Mo-Kα (λ + 0.7107 Å)
temp, °C	25
m, cm^{-1}	1.66
R^a	0.077

$^a R = \sum||F_o| - |F_c||/\sum|F_o|$.

By the same procedure and starting from **7a** with gaseous $HN(CH_3)_2$, **11** as shown in Table II was obtained.

6-Methyl-6,11-dihydro-11-(aminoacetyl)dibenzo[*c*,*f*]-[1,2,5]thiadiazepine 5,5-Dioxide Hydrochloride (39). A mixture of **9a** (6.3 g, 0.019 mol), toluene (10 mL), and NaN_3 (3 g, 0.019 mol) in water (12 mL) with Aliquat 356 (0.8 g, 0.02 mol) was stirred for 16 h. The mixture was filtered, the insoluble solid was collected, washed with water and dried: the crude azido derivative was obtained. It was then suspended in methanol (400 mL), treated with 10% palladium–charcoal catalyst (1 g) and hydrogenated at atmospheric pressure and room temperature until uptake of hydrogen ceased (8 h). After filtration to remove the catalyst, the solution was evaporated under vacuum. The residue was dissolved in chloroform and saturated with gaseous HCl. The precipitate was then collected, dried, and crystallized from 2-PrOH to yield 3.1 g (46%) of **39**: mp 242–244 °C dec; IR (Nujol) 1693 cm^{-1}; ^1H NMR (DMSO-d_6) 3–4.1 (m, 5 H, CH_3 + CH_2), 7.3–8.1 (m, 8 H, arom). Anal. ($C_{15}H_{15}N_3O_3S\cdot HCl$) C, H, N.

X-ray Crystal Structure Determination. A single crystal of **12** with approximate dimensions 0.3 × 0.2 × 0.1 mm was mounted on a Enraf-Nonius CAD4 X-ray diffractometer. A summary of the crystallographic data is reported in Table V. Unit-cell parameters were determined from angular settings of 25 carefully centered reflections. Intensities were corrected for Lorentz and polarization effects. The intensities of three standard reflections were monitored periodically for stability control during data collection. A total of 2470 reflections were collected in the range 5° < 2θ < 45°. A total of 1126 reflections with $I > 3\sigma(I)$ were used in the structure determination. The structure was solved by direct methods of SHELX76 that show all the non-hydrogen atoms.[28] Refinement was performed by means of the full-matrix least-squares methods of SHELX76. Hydrogen atoms were introduced in calculated positions, and their parameters were refined successively. Isotropic thermal parameters were used for carbon and hydrogen atoms, while sulfur, oxygen, and nitrogen atoms were refined anisotropically.

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Supplementary Material Available: Tables listing positional parameters for hydrogen and non-hydrogen atoms, atomic thermal parameters, bond distances, bond angles, and possible hydrogen bonds (6 pages). Ordering information is given on any current masthead page.

(28) Sheldrick, G. M. *SHELX76, Program for Crystal Structure Determination*; University of Cambridge: Cambridge, U.K. 1976.

Novel Tricyclic-α-alkyloxyphenylpropionic Acids: Dual PPARα/γ Agonists with Hypolipidemic and Antidiabetic Activity

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Synthesis and structure–activity relationships of tricyclic α-ethoxy-phenylpropionic acid derivatives guided by in vitro PPARα and PPARγ transactivation data and computer modeling led to the identification of the novel carbazole analogue, **3q**, with dual PPARα (EC_{50} = 0.36 μM) and PPARγ (EC_{50} = 0.17 μM) activity in vitro. Ten days treatment of db/db mice with **3q** improved the insulin sensitivity, as measured by OGTT, better than that seen with both pioglitazone and rosiglitazone treatment, suggesting in vivo PPARγ activity. Likewise, **3q** lowered plasma triglycerides and cholesterol in high cholesterol fed rats after 4 days treatment, indicating in vivo PPARα activity. Investigations of the pharmacokinetics of selected compounds suggested that extended drug exposure improved the in vivo activity of in vitro active compounds.

Introduction

Type 2 diabetes is a metabolic disease characterized by insulin resistance, hyperglycaemia, and often hyperlipidemia. Untreated type 2 diabetes leads to several chronic diseases such as retinopathy, nephropathy, neuropathy, and cardiovascular diseases,[1] the latter leading to increased mortality. Two classes of compounds known as the thiazolidinediones (TZDs) and the fibrates were empirically discovered decades ago to possess the ability to lower blood glucose and lipids in rodent models of insulin resistance and hyperlipedimia, respectively. In humans, fibrates are effective at lowering serum triglycerides and raising HDL cholesterol levels, primarily through increased clearance and decreased synthesis of triglyceride-rich VLDL.[2] Fibrates have been shown to slow the progression of atherosclerosis and reduce the number of coronary events in secondary prevention studies and in patients with normal levels of LDL cholesterol and lately in diabetic patients.[3–7] Interestingly, improvement in glucose tolerance in type 2 diabetic patients has also been shown with clofibrate and bezafibrate.[8–10] Furthermore, fibrates have been reported to reduce weight gain in rodents without effects on food intake.[11] This is of interest since obesity is a major risk factor for the development of type 2 diabetes. Similarly, clinical trials have shown that the TZDs lower blood glucose and insulin levels and improve insulin sensitivity, but they have only marginal effects on plasma lipids in type 2 diabetic patients.[12] The magnitude of the blood glucose lowering effect corresponds to a lowering of HbA_{1c} of 1–1.9% in responders, which typically accounts for 50–70% of the patients.[12] Paradoxically, the improvement in insulin sensitivity is accompanied with a body weight gain, which correlates to the effectiveness of the treatment.[12,13] This limited, although significant, improvement in insulin sensitivity in type 2 diabetic patients undergoing TZD (pioglitazone or rosiglitazone) treatment warrants the development of novel and more effective treatments.

The recent identification of the nuclear receptor peroxisome proliferator activated receptor-γ (PPARγ) and PPARα as being the primary targets for the normoglycaemic TZDs and the lipid lowering fibrates, respectively, has provided new opportunities for the identification of novel compounds for the treatment of type 2 diabetes.[14,15] The successful identification of novel PPARγ selective agonists with good blood glucose lowering activity, using in vitro PPAR receptor binding and in vitro activation screening, has already been described.[16–18]

Despite the growing evidence of the positive effects of fibrate (PPARα agonist) treatment in type 2 diabetic patients, most reports have been on the identification of selective PPARγ agonists. Only a few reports have dealt with selective PPARα agonists,[15] and even fewer compounds have been reported to have both PPARγ and PPARα agonist activity,[19–21] e.g., KRP-297 and (–)-DRF2725/NNC61-0029. We therefore decided to investigate if such dual activating receptor agonists would have improved in vivo efficacy over the aforementioned PPAR subtype selective agonists. The aim of this work was therefore to identify compounds with full efficacy and equal potency on PPARα and PPARγ receptors and to characterize such compounds in animal models predictive of clinical activity.

The non-TZD alkoxy-propionic acid class of insulin sensitizers was chosen as the chemical lead, as this functional group would be less prone to racemization

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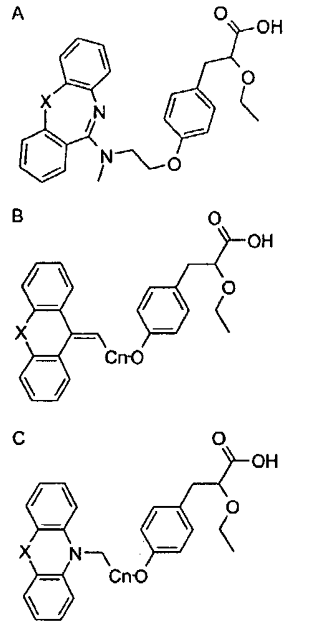


Figure 1. Graphical illustration of the chemical structure evolution of the program.

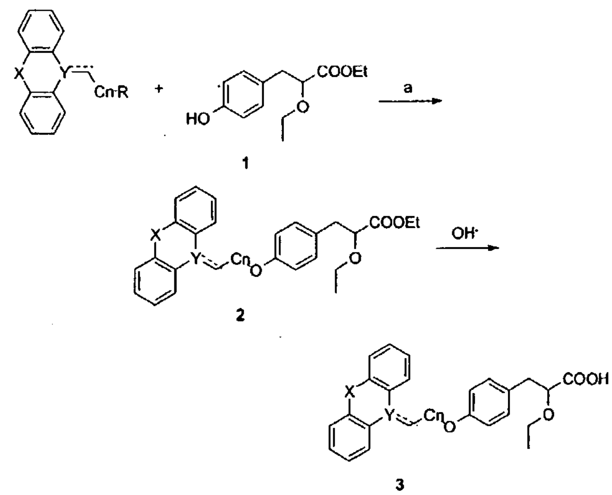
compared to TZD, which undergoes complete racemization under physiological conditions.[22] This was important since only the (*S*)-enantiomers of the TZDs bind to the receptor with high affinity.[23] Further, recent reports have suggested very potent in vitro and in vivo activities of the alkoxy-propionic class of compounds.[24-27]

The present paper reviews the in vitro transactivation activity of the compounds obtained by initially combining structural elements from rosiglitazone (Table 2) and the ethoxy-propionic acid moiety with tricycles to give the lead structure A (Figure 1). These compounds were further revised to compounds B after replacement of the methylamino with a methine group. Finally, the introduction of a nitrogen atom as an attachment point in the tricyclic moiety gave the compounds C, which were both potent and efficacious PPARα and PPARγ agonists in vitro. In vivo experiments with selected compounds suggested that continued drug exposure was critical to the magnitude of the improvement in insulin sensitivity. The carbazole analogue **3q** (Table 2) was identified as having improved insulin sensitizing and lipid lowering effects in vivo compared to rosiglitazone and pioglitazone due to potent intrinsic PPARα and PPARγ activity combined with good pharmacokinetics.

Chemistry

Most of the desired compounds were synthesized by alkylation of ethyl 2-ethoxy-3-(4-hydroxyphenyl)-propionate (**1**) (synthesized by a method analogous to the procedure published by Haigh et al.[28]) with the appropriate tricyclic-alcohol under Mitsunobu conditions using either triphenylphosphine and diethyl azodicarboxylate (DEAD) or tributylphosphine and 1,1′-(azodicarbonyl)dipiperidine (ADDP) as shown in Scheme 1 (procedures A and B). Alternatively, the alkylation was performed with the mesylate or the alkyl halide as in procedures C and D. Aqueous sodium hydroxide hydrolysis of **2** in ethanol gave the target products **3**. The more interesting compounds were synthesized as their

Scheme 1



Procedure
A: R = OH, a: PPh_3, DEAD, THF. (**2e, 2h, 2i, 2j, 2m, 2n, 2o, 2p**)
B: R = OH, a: PBu_3, ADDP, benzene. (**2q, 2r, 2s**)
C: R = $OS(O)_2CH_3$, a: K_2CO_3, DMF. (**2c, 2f, 2k, 2l**)
D: R = Br, a: K_2CO_3, DMF. (**2d, 2g**)

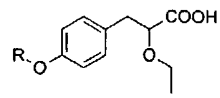
pure (*S*)-enantiomers by using the pure (*S*)-isomer of **1**. The basic hydrolysis of the esters (**2**) leading to the final compounds **3p**, **3q**, **3r**, and **3s** did not cause any measurable recemization. Compounds for in vivo testing in the db/db mouse model of type 2 diabetes and pharmacokinetic measurements in rats were converted to either the lysine or the arginine salts.

Results and Discussion

The aim of the present work was to identify compounds with full efficacy and equal potency on PPARα and PPARγ receptors. In vitro receptor transactivation assays with the binding domains of each of the two PPAR receptor subtypes were used as the primary screening tool in that effort. A similar screening strategy had previously been used in the search for PPARγ selective agonists.[16] To compare the efficacy of compounds from test to test, WY14643 and rosiglitazone were used as reference agonists in the PPARα and PPARγ transactivation assays, respectively. Maximum obtained fold activation with the reference agonist (approximately 20-fold with WY14643 in PPARα, and 120-fold with rosiglitazone in PPARγ) was defined as 100%.

By combining tricyclic ring fragments with structural elements from rosiglitazone (Table 2) and from ethoxy-propionic acids[27] the general lead structure A (Figure 1) was obtained. Selecting either the dihydro-dibenzo-cycloheptene or the dibenzothiazepine as the tricycle gave the specific compounds **3a** and **3b**, respectively. Compound **3a** was a potent and selective PPARγ partial (78%) agonist (Table 1), whereas **3b** was equally potent and efficacious (41−68%) on both PPARα and PPARγ. Although the potency and efficacy was too low to meet our criteria for further investigations, this initial data showed us that it probably would be possible to design compounds with combined PPARα and PPARγ activity. Consequently, additional tricyclic analogues were designed and synthesized. Substitution of the methylamine in **3a** with carbon (methine) gave **3c**. However,

Table 1. In Vitro hPPARα and hPPARγ Transactivation of Novel Racemic Test Compounds[a]



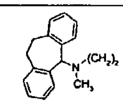
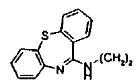
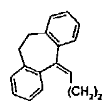
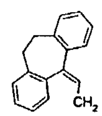
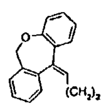
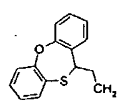
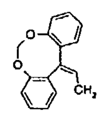
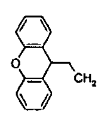
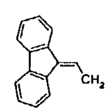
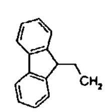
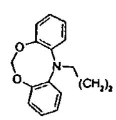
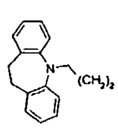
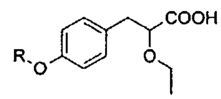
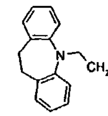
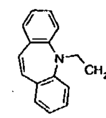
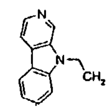
		In vitro activation			
		hPPAR α		hPPAR γ	
Compound no	R	EC_{50}±SD, μM	% max±SD[b]	EC_{50}±SD, μM	% max±SD[c]
3a			12.3±7.2	1.7±1.1	78.3±17.4
3b		7.5±3.4	68.0±16.8	9.8±5.0	41.3±8.5
3c			16.7±4.7	16.7±1.2	32.7±10.8
3d			15.7±4.5	12.6±5.5	80.0±10.1
3e		12.4±4.3	40.3±12.5	12.4±4.8	80.3±15.0
3f		9.0±3.3	69.0±32.0	3.7±1.2	101.0±11.5
3g			17.0±1.7	3.4±1.4	85.7±21.6
3h		9.6±3.0	55.0±12.1	0.46±0.13	117.3±34.5
3i		12.5±6.0	88.0±34.1	12.1±4.0	78.7±11.6
3j		11.7±4.2	84.7±27.4	0.49±0.071	127.0±24.3
3k		1.2±0.58	89.3±26.5	1.2±0.41	106.0±2.6
3l			11.7±3.2	0.76±0.28	108.7±10.1

Table 1. (Continued)



		In vitro activation			
		hPPAR α		hPPAR γ	
Compound no	R	EC_{50}±SD, μM	% max±SD[b]	EC_{50}±SD, μM	% max±SD[c]
3m			21.8±9.4	0.75±0.35	126.5±11.1
3n		9.5±2.1	24.0±2.9	0.76±0.13	125.8±15.5
3o		0.67±0.46	108.7±33.9	0.091±0.073	113.0±11.5

[a] Compounds were tested in at least three separate experiments in five concentrations ranging from 0.01 to 30 μM. EC_{50}s were not calculated for compounds producing transactivation lower than 25% at 30 μM. [b] Fold activation relative to maximum activation obtained with WY14643 (approximately 20-fold corresponded to 100%) and with [c]rosiglitazone (approximately 120-fold corresponded to 100%).

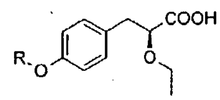
3c was still a selective PPARγ partial agonist but much less potent than **3a**. Changing the chain length from three to two carbon atoms, as in **3d**, slightly increased the PPARγ potency and efficacy but did not improve the PPARα efficacy. Substituting a carbon for an oxygen atom in the tricyclic ring of **3c** gave **3e**, which had some PPARα and PPARγ activity. Further insertion of oxygen atoms in the tricyclic ring of **3d** as in **3g** did not improve PPARα activity but retained the PPARγ activity. Replacing the nitrogen and the sulfur atoms in **3b** with sulfur and oxygen gave a compound, **3f**, with a quite similar in vitro profile.

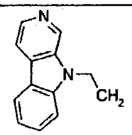
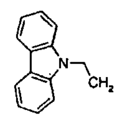
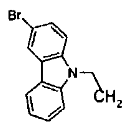
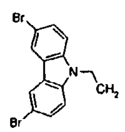
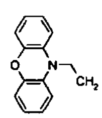
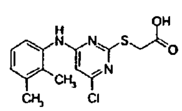
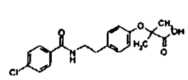
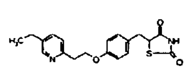
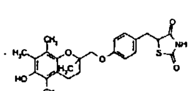
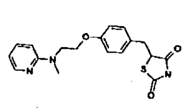
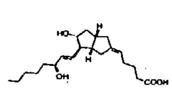
At this point it was decided to use molecular modeling in an attempt to improve the PPARγ potency of the compounds. Using the X-ray structure of rosiglitazone in complex with the binding domain of the PPARγ receptor,[29] the available pocket around the pyridine ring in rosiglitazone was calculated with a Grid protocol using a water probe, Figure 2. These calculations showed that this part of the pocket was rather narrow and that a planar ring system would be preferred. Therefore, rather than expanding the tricyclic ring system, it was decided to make the center ring smaller as in **3h**, **3i**, and **3j**. Two of the three compounds (**3h** and **3j**) were in fact more potent PPARγ agonists than the previous compounds **3a–g**. Furthermore, compounds **3h** and **3j** were full PPARγ but partial and weak PPARα agonists. A similar type of modeling calculation could not be made on the PPARα receptor, as the X-ray structure of this receptor protein was not available.

In an attempt to improve the PPARα potency and efficacy, a series of tricyclic analogues was designed with a nitrogen in the center ring used as the attachment point, **3k–o**. Nitrogen was chosen since that would give a geometry at the attachment point more similar to that seen in the potent PPARγ analogues **3h** and **3j** than in the less potent **3i**. The nitrogen analogue of **3g**, **3k**, was close to having the desired profile. The compound was equally potent on PPARα and PPARγ (EC_{50} = 1.2 μM); it was a full PPARγ agonist and an almost full PPARα (89%) agonist. The nitrogen analogues of **3c** and **3d**, respectively **3l** and **3m**, were both, as predicted, potent full PPARγ agonists, but they had only low PPARα efficacy. A breakthrough was achieved with the planar β-carboline analogue **3o**, which was a potent and full agonist on both PPARα and PPARγ. Despite a 10-fold difference in potency in favor of PPARγ, it was decided to make the pure (*S*)-enantiomer (**3p**, Table 2). The initial lead optimization had, until this point, been carried out on racemic mixtures, but the promising results with **3o** prompted us to continue with pure enantiomers. From the literature,[27,30] it was known that the (*S*)-enantiomer was the active form, which we later confirmed with our own compounds (data not shown).

Compound **3p** showed the expected increase in potency compared to **3o** on both PPARα and PPARγ. In fact, **3p** (Table 2) was approximately 10 times more potent on PPARγ than rosiglitazone. Replacement of the β-carboline ring system with the equally planar carbazole ring system gave the compound **3q**, which had the desired dual PPARα/PPARγ activity profile. Interestingly, a closely related carbazole containing thiazolidinedione (TZD) analogue had previously been reported not to have any glucose lowering effect in vivo.[31] To understand the differences between the ethoxypropionic acid **3q** and the TZD analogue, both compounds were docked into the PPARγ receptor-binding domain. The results showed that the thiazolidinedione moiety was slightly smaller than the ethoxypropionic acid group, resulting in a slightly shorter molecule, which hindered either the carbazole moiety in reaching the lipophilic pocked at the right angle or the TZD in making inter-

Table 2. In Vitro hPPARα, hPPARγ, and hPPARδ Activation of Standard Compounds and Pure (*S*)-Enantiomeric Test Compounds[a]



Compd no	R	In vitro activation: hPPARα EC$_{50}$±SD, μM	hPPARα % max±SD	hPPARγ EC$_{50}$±SD, μM	hPPARγ % max±SD	hPPARδ EC$_{50}$±SD, μM	hPPARδ % max±SD[b]
3p		0.14±0.054	166.0±22.9	0.011±0.0029	116.0±4.6		5.0±0
3q		**0.36±0.16**	**140.4±11.6**	**0.17±0.081**	**108.1±17.6**		3.0±0
3r		2.4±0.25	82.0±7.9	0.33±0.29	109.7±9.3		2.0±0
3s		0.22±0.099	123.5±27.9	0.044±0.029	118.3±14.4		15.5±0.7
(-) DRF 2725		3.21±1.09	96.8±27.5	0.57±0.20	117.2±12.0		7.0±0.8
WY 14643		12.6±1.1	100	29.3±4.3	22.0±2.8		6.0±6.0
bezafibrate		35.7±10.2	95.3±23.7	73.5±7.6	25.0±4.6	102±17.1	91.0±39
pioglitazone		6.68±2.33	57.8±19.5	0.97±0.14	90.8±7.4		1.0±0
troglitazone			14.7±4.9	0.98±0.22	91.5±9.7		1.0±0
rosiglitazone		4.1±1.4	43.0±7.5	0.16±0.015	100		7.0±5.0
carbacyclin		0.34	28[c]		13[c]	2.41±0.78	100

[a] See Table 1. [b] Fold activation relative to maximum activation obtained with carbacyclin (approximately 250-fold corresponded to 100%). [c] One experiment.

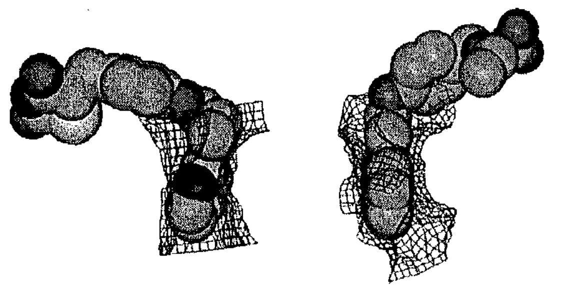
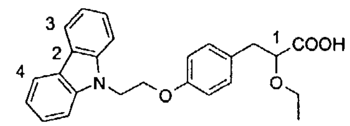


Figure 2. Available pocket around the pyridine ring (left) and around the tricyclic ring (right) in the X-ray structures of the PPARγ receptor binding domain in complex with either rosiglitazone[29] or **3q**, respectively. The available pocket calculated with Grid using a water probe is shown as a blue grid at the energy level 0 kcal/mol, and ligands are shown as space-filling models.

Table 3.



	X-ray structure (Å)	no. of conformations within 2.8 kcal/mol[a]		
	3q	**3q**	**3i**	**3j**
distance 1–2 (9.6–11.6 Å)	10.6	137	25	31
distance 1–3 (10.5–12.5 Å)	11.5	110	7	19
distance 1–4 (10.5–12.5 Å)	11.5	74	0	5
distance 1–2, 1–3, and 1–4		74	0	5

[a] Number of conformations within 2.8 kcal/mol which fulfill distance criteria observed in the crystal structure of the PPARγ receptor binding domain in complex with **3q**.

actions with AF-2 amino acids (data not shown; see also Table 3). Alternatively, poor pharmacokinetic properties of the TZD could explain the lack of in vivo activity.

None of the compounds **3p**, **3q**, **3r**, or **3s** displayed any in vitro activity on the PPARδ receptor subtype (Table 2) or on the RXRα receptor (data not shown). Activity at other nuclear receptors was not investigated.

To further understand the SAR generated in this series of tricyclic analogues and to characterize the receptor interaction, a crystal structure of **3q** in complex with the PPARγ receptor protein was generated. The crystallographic structure of **3q** in complex with the PPARγ receptor binding domain was determined to 2.5 Å resolution using overnight soaking. A bound ligand molecule was found in one of the independent PPARγ receptor molecules. The examination of the ligand–receptor interactions revealed the following observations:

The 2-ethoxypropionic acid in **3q** showed well-defined electron density (Figure 3). The four hydrogen bonds between the propionic acid group of **3q** and the PPARγ receptor protein (Figure 4 and table X in Supporting Information) had all previously been reported to be involved in hydrogen bond formation in other PPARγ receptor/ligand complexes,[29,32,33] e.g., rosiglitazone as shown in Figure 4. The ethoxy group gave further hydrophobic interactions with Phe 282.

The phenyl group of the ethoxy-phenyl moiety in **3q** also showed well-defined electron density. This part formed van der Waals interactions with Met 364, Cys

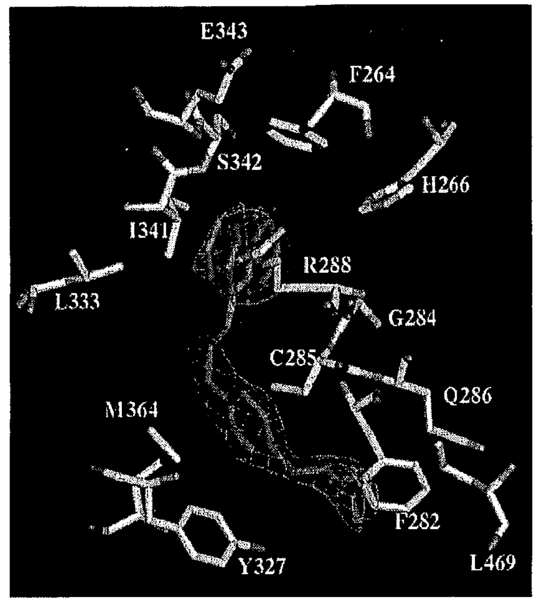


Figure 3. Crystal structure of the PPARγ receptor binding domain in complex with **3q**. The ligand carbon atoms are shown in magenta. Amino acids of the PPARγ receptor neighboring the ligand are shown with green-colored carbon atoms and with their residue type and sequence numbers written in yellow. Also shown, as a chicken wire net representation, are the experimental SigmaA weighted electron density maps: in blue, the map with the coefficients $2F_o - F_c$ at 1σ cutoff; and in brown, the negative side of the $F_o - F_c$ coefficients map at 3σ cutoff.

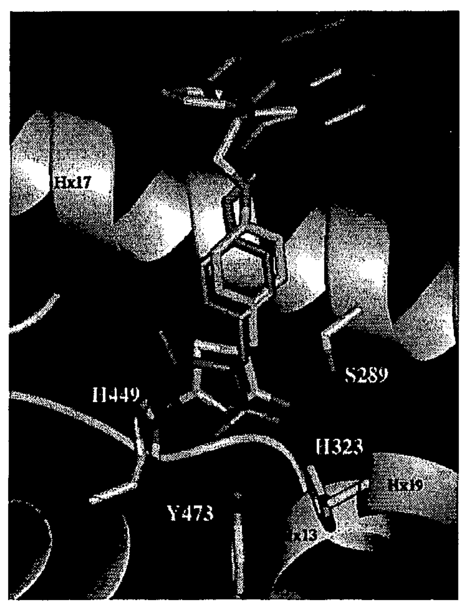


Figure 4. Sketch of the relative positions of **3q** (magenta) and rosiglitazone (green[29]) bound to the PPARγ receptor binding domain. Indicated are also the residues involved in hydrogen binding to **3q**, with their residue type and sequence numbers written in yellow, and written in black, the numbers of the helices in the active site region.

285, Tyr 327, and at a somewhat greater distance, 4.3 Å, the Leu 330 (Figure 3).

The carbazole ring, situated in the large hydrophobic pocket of the PPARγ receptor protein, showed, however, less well-defined electron density. The electron density from the ethyl group atoms connecting the phenoxy and

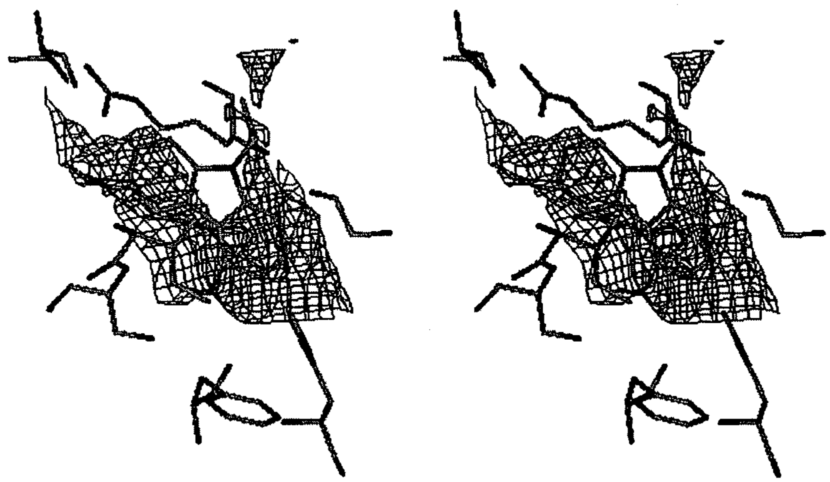


Figure 5. Stereoview of the crystal structure of the PPARγ receptor binding domain in complex with **3q**. The blue grid shows the interactions between the active site and an aromatic probe at level −2.3 kcal/mol.

the carbazole ring was missing, moreover there were even some negative difference electron density regions seen close to this bond. The electron density around the ring system was also somewhat weak. In addition, for this part of the ligand, the temperature factors were also slightly higher, above 70 Å^2, compared to the rest of the molecule that had temperature factors below 70 Å^2. This lack of well-defined electron density indicated than the carbazole region of **3q** was relatively mobile, or alternatively had more than one conformation that was not easily interpreted. To investigate the available cavity and the interactions between the aromatic rings and the amino acids in the binding pocket, Grid calculations with an aromatic probe were performed. During these calculations the ligand was neglected (Figure 5). From these calculations it could be seen that the tricyclic ring system was located where favorable interactions between an aromatic group and the receptor were observed, mainly Gly 284, Ile 341, Arg 288, Ser 342, Phe 264, His 266, and Leu 333 (Figures 3 and 5). Furthermore, the available binding pocket was, as predicted (see also Figure 2), narrow but large enough for sideway movements possibly explaining the less well-defined electron density.

The importance of the attachment atom (carbon vs nitrogen) and bond type (single vs double) to the tricyclic ring system, which had been experimentally observed (**3c** vs **3l**; **3d** vs **3m**; **3i** vs **3j** vs **3q**; Tables 1 and 2), was also investigated using the crystal structure and modeling. The ligands **3q**, **3i**, and **3j** were chosen as model compounds for these calculations. In this series, both **3q** and **3j** were potent PPARγ agonists while **3i** was a very weak PPARγ agonist. Two geometric parameters, which were considered important for the ligands to be able to adopt the shape of the binding pocket in the PPARγ receptor, were the distance between the carboxylic acid and the tricyclic ring system, and the u-shape of the molecule. To analyze the possibility for **3q**, **3i**, and **3j** to adopt a shape which was comparable with the shape of the binding pocket, the distances between the carbon atom connected to the carboxylic acid group and three different atoms in the tricyclic ring system were calculated and compared to the distance measured in the X-ray structure of the PPARγ receptor binding domain in complex with **3q**, Table 3. To sample the possible conformations, conformational analyses were performed using the MMFF force field[34−37] and a systematic pseudo Monte Carlo search in MacroModel 7.0.[38,39] The results showed (Table 3) that both **3q** and **3j** could adopt conformations, which fulfill all three distance criteria for conformations within 2.8 kcal/mol, while **3i** could not. This means that although the tricyclic ring system in **3i** could reach the lipophilic binding pocket of the receptor (distance 1−2, Table 3), the carbon−carbon double bond prevented the ligand from adopting the necessary curved conformation (distance 1−4).

In the male db/db mouse, our primary in vivo model for improvement of insulin sensitivity, the two marketed PPARγ agonists, rosiglitazone (Avandia) and pioglitazone (Actos), showed dose-related reduction of nonfasted blood glucose (BG) when dosed orally by gavage once daily for 7 days (Table 4, Figure 6a). The maximum obtained BG reduction with rosiglitazone maleate, when treated with the doses 0.2, 0.6, 2.0, and 6.0 mg/kg, was 35% compared to vehicle treated animals (53% normalization compared to lean db/+ mice; see Experimental Section for calculation). The less potent pioglitazone gave a 53% reduction (80% normalization) when dosed at 3.0, 10, 30, and 100 mg/kg. Both compounds also showed a dose-related reduction of nonfasted plasma insulin and a non-dose-related reduction in TG (Table 4). TG data were, however, not used as selection criteria, since the observed TG lowering effect of rosiglitazone and pioglitazone had not been found in clinical trials.

The animals were dosed for a further 2 days (a total of 9 days treatment), after which an oral glucose tolerance test (OGTT) was performed. The reduction of the blood glucose area under the curve (AUC_{glu}), when the animals were given an oral glucose dose (3 g/kg), was considered to be a more direct measure of the improved insulin sensitivity of the compounds. Surprisingly, rosiglitazone only reduced the AUC_{glu} by 16% compared to vehicle treated animals (25% normalization compared to lean db/+ mice), whereas pioglitazone

Table 4. In Vivo Efficacy in Male db/db Mice after Oral Treatment for 7–9 Days[a]

compd no.	BG[b] ED_{50}, mg/kg	BG % max reduction	TG[c] ED_{50}, mg/kg	TG % max reduction	insulin ED_{50}, mg/kg	insulin % max reduction	AUC_{glu}[d] ED_{50}, mg/kg	AUC_{glu} % max reduction
3k	8.11 ± 1.04	17 ± 8	18.28 ± 1.23	13 ± 6	ND	NE	6.57 ± 0.78	20 ± 5
3p	0.33 ± 0.80	46 ± 4	2.68 ± 0.62	50 ± 2	7.74 ± 0.89	77 ± 4	3.32 ± 0.71	12 ± 11
3q	0.27 ± 0.94	58 ± 7	0.52 ± 0.56	52 ± 10	0.34 ± 0.78	85 ± 3	0.40 ± 0.62	55 ± 13
rosiglitazone	0.87 ± 0.62	35 ± 7	0.14 ± 0.91	58 ± 2	0.07 ± 1.27	43 ± 9	0.77 ± 0.62	16 ± 9
pioglitazone	2.62 ± 0.19	53 ± 3	26.74 ± 0.60	21 ± 5	15.05 ± 0.97	54 ± 10	12.09 ± 0.79	39 ± 8

[a] Male db/db mice ($n = 6$) were treated once a day by oral gavage for 9 days. Compound **3k** was tested at the doses 0.3, 1.0, 3.0, and 10.0 mg/kg/day; **3p** at 1.0, 3.0, 10.0, and 30 mg/kg/day; **3q** at 1.0, 3.0, 5.0, and 20 mg/kg/day; rosiglitazone at 0.2, 0.6, 2.0, and 6.0 mg/kg/day; and pioglitazone at 3.0, 10.0, 30.0, and 100.0 mg/kg/day. ED_{50} values were calculated via nonlinear regression using GraphPad PRISM 3.02 and are expressed as mean ± SEM. "% max reduction" is the maximum achieved reduction relative to vehicle treated control group ± SEM. [b] Nonfasting blood glucose after 7 days treatment. [c] Nonfasting triglycerides after 7 days treatment. [d] Area under blood glucose time curve after OGTT on the 9th day of treatment.

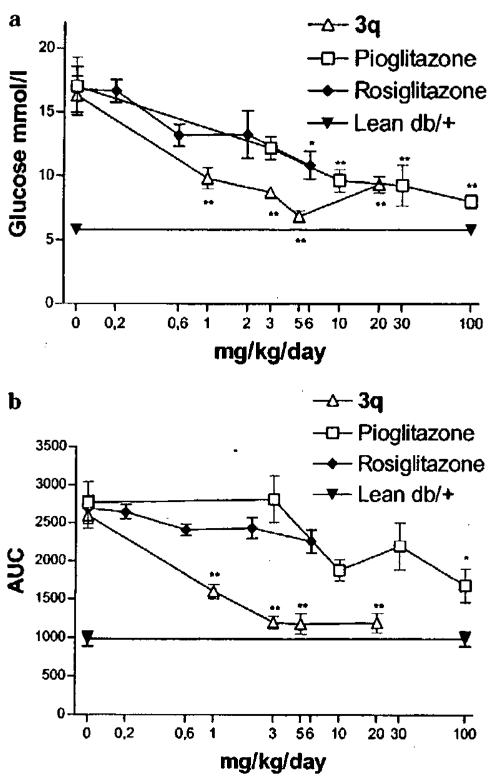


Figure 6. (a) Dose-related reduction of the nonfasted blood glucose in male db/db mice ($n = 6$) treated for 7 days with **3q**, rosiglitazone, and pioglitazone orally once a day. Values are expressed as mean ± SEM. * represents $P < 0.05$, ** $P < 0.01$ using one way ANOVA and Dunetts multiple comparison test. (b) Dose-related reduction of the area under the blood glucose concentration (AUC_{glu}) vs time curve after oral glucose tolerance test (OGTT) in male db/db mice ($n = 6$) treated for 9 days with **3q**, rosiglitazone, and pioglitazone orally once a day. Values are expressed as mean ± SEM. * represents $P < 0.05$, ** $P < 0.01$ using one way ANOVA and Dunetts multiple comparison test.

reduced the AUC_{glu} by 39% (61% normalization) (Table 4, Figure 6b).

Three compounds identified by the in vitro activation screening, **3k**, **3p**, and **3q**, were tested in db/db mice. Despite the quite impressive in vitro activity, **3k** only produced a very modest dose-related reduction in BG (17% compared to vehicle and 25% normalization) and AUC_{glu} (20% of vehicle and 31% normalization) when dosed at 0.3, 1.0, 3.0, and 10 mg/kg of the lysine salt. Compound **3p** gave a better reduction in BG (46% of vehicle and 77% normalization) but surprisingly had very little effect on the AUC_{glu} (12% of vehicle and 22% normalization) when dosed at 1.0, 3.0, 10, and 30 mg/kg of the arginine salt. However, a significant effect was obtained with **3q**. When dosed at 1.0, 3.0, 5.0, and 20 mg/kg of the **3q** arginine salt, a dose-related reduction of both BG (58% of vehicle and 78% normalization) and AUC_{glu} (55% of vehicle and 89% normalization) was obtained (Table 4 and Figure 6a,b).

Table 5. Single Dose Rat Pharmacokinetics after iv and po Administration of Selected Compounds[a]

compd no.	C_{max} po,[b] ng/mL	AUC_{po},[c] (ng × min)/mL	F_{po},[d] %	CL[e] mL/min/kg	V_{ss}[f] L/kg	$T_{(1/2)po}$[g] min
3k	730	ND	63	13.7	0.76	ND
3p	527	79523	61	12.7	0.88	162
3q	4430	8643717	>100	0.75	0.73	1332
rosiglitazone	4420	873922	83	2.1	0.38	182
pioglitazone	4655	1753075	112	1.4	0.22	228

[a] Rats were given either a single dose iv (1.2 mg/kg) ($n = 8$) or a single dose po (2.2 mg/kg) ($n = 8$) of each of the test compounds. At each of the time points (5, 15, 30, 60, 90, 120, 240, and 360 min), one animal was sacrificed, and blood samples were analyzed for compound plasma concentration. [b] Maximum plasma concentration after oral dosing. [c] Estimated area under the plasma-concentration time curve after oral dosing. [d] Oral bioavailability. [e] Clearance. [f] Volume of distribution during steady state. [g] Oral half-life.

The somewhat surprising db/db mouse results made us investigate the pharmacokinetics of the compounds to see if that could explain the differences in the in vivo effects.

Rats were given either a single dose iv (1.2 mg/kg) ($n = 8$) or a single dose po (2.2 mg/kg) ($n = 8$) of each of the test compounds. One animal was sacrificed at each of the time points (5, 15, 30, 60, 90, 120, 240, and 360 min), and blood samples were analyzed for compound plasma concentration. Data showed that the test compounds had very different pharmacokinetic parameters (Table 5). The two standard compounds rosiglitazone and pioglitazone had approximately the same maximum plasma concentration ($C_{max} \sim 4400$ (ng × min)/mL) when given the same oral dose. The estimated areas under the curve (AUC_{po}) were, however, quite different, with pioglitazone having an AUC_{po} twice as big as that of rosiglitazone (Table 5). The higher pioglitazone exposure was a consequence of the lower clearance rate, resulting in a longer plasma half-life ($t_{1/2po} = 228$ min for pioglitazone and 182 min for rosiglitazone).

The essentially inactive compound **3k** had much lower C_{max} and AUC_{po}values; the latter could not be estimated due to the few data points resulting from the short half-life. The other low efficacy compound **3p** also showed low C_{max} and low AUC_{po} compared to pioglitazone (Table

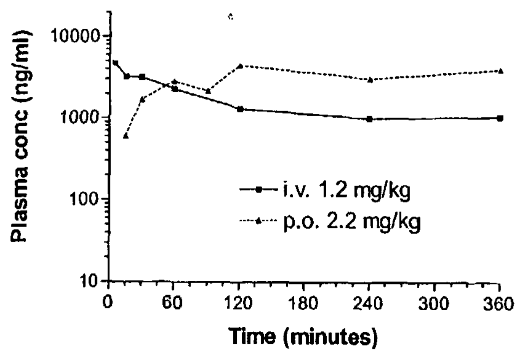


Figure 7. Plasma drug concentration versus time curves obtained from rats dosed with **3q** orally by gavage and intravenously. At each time point, one rat was sacrificed and drug plasma concentration was measured.

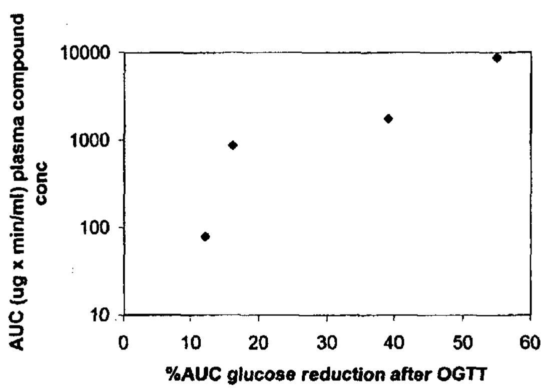


Figure 8. Correlation between area under the plasma compound-concentration versus time curve (AUC_{compd}) after 2.2 mg/kg po treatment (**3p**, **3q**, rosiglitazone, and pioglitazone, Table 5) and the percent maximum reduction in area under the plasma glucose-concentration versus time curve (%AUC_{glu}) obtained in db/db mice (Table 4).

5). The carbazole analogue **3q**, which had shown extremely good lowering effects on both BG and on AUC_{glu}, had a C_{max} similar to that of pioglitazone but an even higher (approximately 5 times) AUC_{po}. Again the increased drug exposure was a result of the low clearance and the resulting long plasma half-life ($t_{1/2po}$ = 1332 min). The half-life of **3q** (Figure 7) was further extended by entero-hepatic recirculation, which also accounted for the high oral bioavailability (F_{po}, Table 5).

The db/db mouse and rat pharmacokinetic data in Tables 4 and 5, respectively, suggested that not only was the in vivo potency dependent on the pharmacokinetics of the compounds, but also the efficacy was dependent on the pharmacokinetics. As depicted in Figure 8, the drug exposure (AUC_{po}) of the compounds correlated with their maximum ability to reduce the AUC_{glu} after OGTT.

The data further suggested that it was the extended drug exposure (long drug half-life) that improved the efficacy of the compound in vivo. To investigate this, a separate experiment with rosiglitazone in db/db mice was designed. Two doses (1 and 3 mg/kg/day po) of rosiglitazone were given either once a day (1 × 1 mg/kg and 1 × 3 mg/kg) or over three doses (3 × 0.33 mg/kg and 3 × 1 mg/kg). The results showed that dividing the dose over three times per day improved both the BG lowering and especially the AUC_{glu} lowering ability of rosiglitazone (Table 6). These findings were in accordance with clinical results performed with rosiglitazone in diabetic patients. Data showed that rosiglitazone was more effective in lowering blood glucose and HbA_{1c} when given twice a day than when given once a day.[40]

Table 6. In Vivo Effects of Rosiglitazone in Male db/db Mice after Once-a-Day or Three-Times-a-Day Oral Dosing[a]

	effects after 7 and 9 days dosing			
rosiglitazone dose	BG, mM	% BG red.[b]	AUC_{glu} after OGTT, mM × min	% AUC_{glu} red. after OGTT[c]
vehicle	2150 ± 1.24		2954 ± 208	
1 mg/kg × 1	16.08 ± 1.55	25 ± 7	2551 ± 180	14 ± 6
0.33 mg/kg × 3	11.35 ± 1.31	47 ± 6	2398 ± 338	18 ± 11
3 mg/kg × 1	11.72 ± 1.29	45 ± 6	2141 ± 165	28 ± 6
1 mg/kg × 3	13.06 ± 1.88	39 ± 9	1423 ± 86	51 ± 3

[a] Male db/db mice (n = 6) were treated for 9 days with rosiglitazone at 1 mg/kg or 3 mg/kg total daily dosage, given either once a day or three times daily by oral gavage. [b] Nonfasting blood glucose was measured on day 7, and the percent reduction relative to vehicle treated group calculated. [c] On day 9 an oral glucose tolerance test was performed, and the area under the plasma glucose-concentration time curve was measured and the percent reduction relative to vehicle treated group calculated. Values are expressed as mean ± SEM.

Table 7. Changes in Nonfasted Plasma Triglycerides and Total Cholesterol in High Cholesterol Fed Male Sprague–Dawley Rats after 4 Days Oral Treatment[a]

	TG		total cholesterol	
compd	ED_{50}, mg/kg	% reduction	ED_{50}, mg/kg	% reduction
3q	0.06 ± 0.69	56 ± 6	0.34 ± 0.60	50 ± 6
bezafibrate	16.51 ± 0.53	63 ± 8	16.71 ± 0.57	74 ± 4
rosiglitazone	ND	11 ± 12	ND	24 ± 6

[a] Six-weeks-old male Sprague–Dawley rats (n = 6) were fed on a high cholesterol diet ad libitum (1.25% cholesterol, 0.5% cholic acid) for 10 days. From day 7 to day 10 the animals were dosed orally by gavage once a day (rosiglitazone 10 and 30 mg/kg, bezafibrate 10, 30, 100, and 300 mg/kg, and **3q** 0.1, 0.3, 1.0, and 3 mg/kg). On day 10, nonfasting blood samples were collected and analyzed for triglycerides (TG) and total cholesterol. ED_{50} values were calculated via nonlinear regression using GraphPad PRISM 3.02 and are expressed as mean ± SEM. "% reduction" is the maximum achieved reduction relative to vehicle treated control group ± SEM.

The results suggested that the best improvement of insulin sensitivity would be obtained with drugs that give long compound exposure. This could be obtained by either selecting compounds with long plasma half-lives, as seen with **3q**, or by using sustained release formulations.

To estimate the in vivo PPARα effect of **3q**, we used the high cholesterol fed rat model, which previously had been used to select the clinically effective fibrates.[41] Male Sprague–Dawley rats were fed a high cholesterol diet for 10 days, the last 4 days on once a day oral drug treatment. Nonfasted TG and total cholesterol were measured and calculated as the percent reduction relative to vehicle control. Rosiglitazone, even at a high dose (30 mg/kg po), did not lower plasma TG or cholesterol significantly (Table 7), suggesting that PPARγ receptor activation was not involved in plasma lipid regulation. Bezafibrate, on the other hand, produced the expected dose-related reduction of both TG (63%) and cholesterol (74%) when dosed at 10, 30, 100, and 300 mg/kg po for 4 days. At 100 times lower doses, 0.1, 0.3, 1.0, and 3.0 mg/kg po, **3q** produced a similar dose-related reduction of TG (56%) and cholesterol (50%,

Hz, 2H) 2.94 (bt, $J = 7$ Hz, 2H), 3.35 (m, 1H), 3.60 (m, 1H), 3.81 (t, $J = 7$ Hz, 2H), 3.97 (t, $J = 7$ Hz, 1H), 4.15 (q, $J = 7$ Hz, 2H), 4.27 (t, $J = 7$ Hz, 1H), 6.75 (bd, $J = 7$ Hz, 2H), 6.98–7.25 (m, 10H), 7.34 (2H, s).

2-(9*H*-Xanthen-9-yl)ethanol. To a suspension of 9-xanthenylacetic acid[43] (4.9 g, 21.5 mmol) in toluene (180 mL) was added a mixture of sodium dihydrido-bis(2-methoxyethoxy)-aluminate (60% solution in toluene, 14.5 g, 43.0 mmol) and toluene (10 mL) dropwise over 10 min under an argon atmosphere. The reaction mixture was stirred at ambient temperature for 2 h. The mixture was cooled to 10 °C and decomposed with water (5 mL) and 15% NaOH (40 mL). The toluene layer was separated and the water layer extracted with toluene (2 × 30 mL). The combined toluene solutions were washed with water (2 × 30 mL) and brine (20 mL), dried ($MgSO_4$), and evaporated in vacuo. The residue (5.0 g) was purified by column chromatography (silica gel Fluka 60, 80 g) using chloroform as eluent. This afforded 3.8 g (78%) of the title compound. ^1H NMR ($CDCl_3$) δ 1.50 (bs, 1 H), 1.92 (q, $J =$ 7 Hz, 2 H), 3.55 (t, $J = 6.5$ Hz, 2 H), 4.16 (t, $J = 6.7$ Hz, 1 H), 7.11–7.02 (m, 4 H), 7.25–7.15 (m, 4 H).

Ethyl 2-Ethoxy-3-[4-(2-fluoren-9-ylidene-ethoxy)phenyl]propionate, 2i. From 2-fluoren-9-ylidene-ethanol.[44] Yield 280 mg (60%). ^1H NMR ($CDCl_3$) δ 1.17 (t, $J = 7$ Hz, 3H), 1.22 (t, $J = 7$ Hz, 3H), 2.97 (d, $J = 7$ Hz, 2H), 3.29–3.40 (m, 1H), 3.54–3.66 (m, 1H), 3.98 (t, $J = 7$ Hz, 1H), 4.17 (q, $J = 7$ Hz, 2H), 5.32 (d, $J = 6$ Hz, 2H), 6.87 (t, $J = 6$ Hz, 1H), 6.92 (d, $J = 8$ Hz, 2H), 7.18 (d, $J = 8$ Hz, 2H), 7.22–7.45 (m, 4H), 7.57–7.77 (m, 4H). MS: 428 (M^+), 382, 191 (100%).

Ethyl 2-Ethoxy-3-(4-[2-(9*H*-fluoren-9-yl)ethoxy]phenyl)-propionate, 2j. From 2-(9*H*-fluoren-9-yl)ethanol.[45] Yield 0.20 g (47%). ^1H NMR ($CDCl_3$) δ 1.17 (t, $J = 7$ Hz, 3H), 1.22 (t, $J = 7$ Hz, 3H), 2.46 (q, $J = 7$ Hz, 2H), 2.93 (d, $J = 7$ Hz, 2H), 3.28–3.40 (m, 1H), 3.52–3.65 (m, 1H), 3.90 (t, $J = 7$ Hz, 2H), 3.95 (t, $J = 7$ Hz, 1H), 4.16 (q, $J = 7$ Hz, 2H), 4.15–4.28 (m, 1H), 6.74 (d, $J = 8$ Hz, 2H), 7.11 (d, J0 8 Hz, 2H), 7.25–7.42 (m, 4H), 7.52 (d, $J = 8$ Hz, 2H), 7.78 (d; $J = 8$ Hz, 2H), 7.78 (d, $J = 8$ Hz, 2H). MS 430 (M^+), 384, 299, 193, 179, 165 (100%), 107.

Ethyl 3-(4-(2-(Dibenzo[*b,f*]azepin-5-yl)ethoxy)phenyl)-2-ethoxypropionate, 2n. Yield 172 mg (75%). ^1H NMR ($CDCl_3$) δ 1.11–1.29 (m, 6H), 2.93 (d, $J = 7$ Hz, 2H), 3.25–3.91 (m, 1H), 3.50–3.67 (m, 1H), 3.95 (t, $J = 7$ Hz, 1H), 4.02–4.21 (m, 6H), 6.75 (t, $J = 7$ Hz, 4H), 6.95–7.30 (m, 10 H).

Ethyl 3-(4-(2-(β-Carbolin-9-yl)ethoxy)phenyl)-2-ethoxypropionate, 2o. Yield 1.09 g (76%). ^1H NMR ($CDCl_3$) δ 1.12 (t, $J = 7$ Hz, 3H), 1.21 (t, $J = 7$ Hz, 3H), 2.90 (d, $J = 7$ Hz, 2H), 3.24–3.37 (m, 1H), 3.51–3.62 (m, 1H), 3.91 (t, $J = 7$ Hz, 1H), 4.14 (q, $J = 7$ Hz, 2H), 4.38 (t, $J = 7$ Hz, 2H), 4.80 (t, $J = 7$ Hz, 2H), 6.74 (d, $J = 8$ Hz, 2H), 7.08 (d, $J = 8$ Hz, 2H), 7.28–7.70 (m, 4H), 7.96 (d, $J = 7$ Hz, 1H), 8.15 (d, $J = 8$ Hz, 1H), 8.49 (d, $J = 7$ Hz, 1H).

Ethyl (*S*)-3-(4-(2-(β-Carbolin-9-yl)ethoxy)phenyl)-2-ethoxypropionate, 2p. Using ethyl (*S*)-2-ethoxy-3-(4-hydroxyphenyl)propionate (95.5% ee). Yield 1.67 g (60%). ^1H NMR ($CDCl_3$) δ 1.12 (t, $J = 7$ Hz, 3H), 1.21 (t, $J = 7$ Hz, 3H), 2.90 (d, $J = 7$ Hz, 2H), 3.24–3.37 (m, 1H), 3.51–3.62 (m, 1H), 3.91 (t, $J = 7$ Hz, 1H), 4.14 (q, $J = 7$ Hz, 2H), 4.38 (t, $J = 7$ Hz, 2H), 4.80 (t, $J = 7$ Hz, 2H), 6.74 (d, $J = 8$ Hz, 2H), 7.08 (d, $J = 8$ Hz, 2H), 7.28–7.70 (m, 4H), 7.96 (d, $J = 7$ Hz, 1H), 8.15 (d, $J = 8$ Hz, 1H), 8.49 (d, $J = 7$ Hz, 1H).

General Procedure B. (*S*)-Ethyl 3-(4-(2-Carbazol-9-yl-ethoxy)phenyl)-2-ethoxypropionate, 2q. To an ice cooled solution of 2-(carbazol-9-yl)ethanol (211 mg; 1.0 mmol), (*S*)-ethyl 2-ethoxy-3-(4-hydroxyphenyl)propionate (1) (99.0% ee) (238 mg; 1 mmol), and tributylphosphine (370 μL; 1.5 mmol) in dry benzene (10 mL) was added 1,1′-(azodicarbonyl) dipiperidine (380 mg; 1.5 mmol). The reaction mixture was stirred at 0 °C for 1 h, an additional 10 mL of benzene added, and the reaction mixture was stirred for another 1 h. Heptane (10 mL) was added to the reaction mixture, and the resulting precipitate was removed by filtration. The filtrate was evaporated in vacuo and the residue suspended in heptane. After filtration, the heptane phase was evaporated to dryness. The residue was purified by column chromatography using toluene:ethyl acetate (19:1) as eluent. The title compound was obtained in 385 mg (89%) yield. ^1H NMR ($CDCl_3$) δ 1.15 (t, $J = 7$ Hz, 3H), 1.22 (t, $J = 7$ Hz, 3H), 2.93 (d, $J = 7$ Hz, 2H), 3.32 (m, 1H), 3.57 (m, 1H), 3.93 (t, $J = 7$ Hz, 1H), 4.15 (q, $J = 7$ Hz, 2H), 4.32 (t, $J = 7$ Hz, 2H), 4.70 (t, $J = 7$ Hz, 2H), 6.74 (d, $J = 8$ Hz, 2H), 7.10 (d, $J = 8$ Hz, 2H), 7.27 (m, 2H), 7.50 (m, 4H), 8.12 (d, $J = 8$ Hz, 2H).

The following compounds were made according to procedure B using the appropriate tricyclyl ethanol:

(*S*)-Ethyl 3-(4-(2-(3-Bromo-carbazol-9-yl)ethoxy)phenyl)-2-ethoxypropionate, 2r. From (*S*)-ethyl 2-ethoxy-3-(4-hydroxyphenyl)propionate (1) (95.5% ee). Yield 510 mg (33%). ^1H NMR ($CDCl_3$) δ 1.15 (t, $J = 7$ Hz, 3H), 1.22 (t, $J = 7$ Hz, 3H), 2.94 (d, $J = 7$ Hz, 2H), 3.33 (m, 1H), 3.58 (m, 1H), 3.93 (t, $J = 7$ Hz, 1H), 4.25–4.10 (q, $J = 7$ Hz, 2H), 4.33 (t, $J = 7$ Hz, 2H), 4.70 (t, $J = 7$ Hz, 2H), 6.70 (d, $J = 8$ Hz, 2H), 7.10 (d, $J = 8$ Hz, 2H), 7.27 (m, 1H), 7.40 (d, $J = 8$ Hz, 1H), 7.60–7.47 (m, 3H), 8.05 (d, $J = 8$ Hz, 1H), 8.20 (s, 1H).

(*S*)-Ethyl 3-(4-(2-(3,6 Dibromo-carbazol-9-yl)ethoxy)-phenyl)-2-ethoxypropionate, 2s. From (*S*)-1 (95.5% ee). Yield 774 mg (100%). ^1H NMR ($CDCl_3$) δ 1.15 (t, $J = 7$ Hz, 3H), 1.22 (t, $J = 7$ Hz, 3H), 2.94 (d, $J = 7$ Hz, 2H), 3.31 (m, 1H), 3.58 (m, 1H), 3.94 (t, $J = 7$ Hz, 1H), 4.17 (q, $J = 7$ Hz, 2H), 4.30 (t, $J = 7$ Hz, 2H), 4.80 (t, $J = 7$ Hz, 2H), 6.68 (d, $J = 8$ Hz, 2H), 7.10 (d, $J = 8$ Hz, 2H), 7.40 (d, $J = 8$ Hz, 2H), 7.58 (d, $J = 8$ Hz, 2H), 8.14 (s, 2H).

General Procedure C. Ethyl 3-(4-(2-(10,11-Dihydro-dibenzo[*b,f*]azepin-5-yl)propoxy)phenyl)-2-ethoxypropionate, 2l. A mixture of ethyl 3-(4-hydroxyphenyl)-2-ethoxypropionate (2.26 g, 10.75 mmol), 3-(10,11-dihydro-5*H*-dibenzo-[*b,f*]azepin-5-yl)propanol methane sulfonate (3.55 g, 10.71 mmol), and potassium carbonate (7.65 g, 55.35 mmol) in DMF (75 mL) was heated at 90 °C for 30 h. The cooled reaction mixture was poured into water (500 mL) and extracted with benzene (3 × 100 mL), and the extracts were washed with water (200 mL), dried ($MgSO_4$), and evaporated in vacuo. The residue (4.75 g) was purified by column chromatography on silica gel (Fluka 60, 150 g) using benzene/chloroform 20:1 as eluent to give the title compound in 1.84 g (36%) yield. ^1H NMR ($CDCl_3$) δ 1.15 (t, $J = 7$ Hz, 3H), 1.21 (t, $J = 7$ Hz, 3H), 2.03 (q, $J = 6.4$ Hz, 2H), 2.92 (d, $J = 6.8$ Hz, 2H), 3.14 (s, 4H), 3.33 (m, 1H), 3.59 (m, 1H), 3.87–4.00 (m, 5H), 4.15 (q, $J = 7.2$ Hz, 2H), 6.72 (dt, $J = 8.7$ and 2.2 Hz, 2H), 6.87–6.95 (m, 2H), 7.05–7.15 (m, 8H).

The following compounds were made according to procedure C using the appropriate mesylate.

Ethyl 3-(4-(3-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)propoxy)phenyl)-2-ethoxypropionate, 2c. From 3-(10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)-propanol.[46] Yield 1.5 g (21%). ^1H NMR ($CDCl_3$) δ 0.99 (t, $J = 7$ Hz, 3H), 1.27 (t, $J = 7$ Hz, 3H), 2.69 (q, $J = 7$ Hz, 2H), 3.06 (d, $J = 7$ Hz, 2H), 3.17 (bs, 4H), 3.45 (m, 1H), 3.68 (m, 1H), 4.07 (m, 3H), 4.27 (q, $J = 7$ Hz, 2H), 6.06 (t, $J = 7$ Hz, 1H), 6.85 (d, $J = 6$ Hz, 2H), 7.10–7.35 (m, 10H).

Ethyl 2-Ethoxy-3-(4-[2-(11*H*-5-oxa-10-thia-dibenzo[*a,d*]-cyclohepten-11-yl)ethoxy]phenyl)propionate, 2f. Yield 4.6 g (60%). ^1H NMR ($CDCl_3$) δ 1.16 (t, $J = 7$ Hz, 3H), 1.20 (dt, $J = 0.6$ and 7 Hz, 3H), 2.53–2.80 m, 2H), 2.94 (d, $J = 6.6$ Hz, 2H), 3.34 (dq, $J = 7.0$ and 9.1 Hz, 1H), 3.59 (dq, $J = 7$ and 9.1 Hz, 1H), 3.96 (m, 1H), 4.00 (m, 1H), 4.15 (q, $J = 7$ Hz, 2H), 4.18 (m, 1H), 4.70 (dd, $J = 6.9$ and 8.5 Hz, 1H), 6.80 (t, $J = 7$ Hz, 2H), 6.93 (ddd, $J = 1.5$, 6.8 and 7.8 Hz, 1H), 7.01–7.26 (m, 9H).

Ethyl 3-(4-Dibenzo[*d,g*]dioxazocin-12-yl)-1-propoxy)-phenyl-2-ethoxypropionate, 2k. From 3-(4-dibenzo[*d,g*]-dioxazocin-12-yl)-1-propanol. Yield 2.45 g (42%). ^1H NMR ($CDCl_3$) δ 1.15 (t, $J = 7.2$ Hz, 3H), 1.21 (t, $J = 7.2$ Hz, 3H), 1.92 (q, $J = 5.7$ Hz, 2H), 3.33 (m, 1H), 3.59 (m, 1H), 3.81 (t, $J = 5.7$ Hz, 2H), 3.97 (t, $J = 5.7$ Hz, 3H), 4.15 (q, $J = 7.2$ Hz, 2H), 5.71 (s, 2H), 6.75 (dt, $J = 8.4$ Hz, 2H), 7.20–6.95 (m, 10H).

General Procedure D. Ethyl 3-(4-[2-(10,11-Dihydro-dibenzo[*a,d*]cyclohepten-5-ylidene)ethoxy]phenyl)-2-

ethoxypropionate, 2d. A mixture of **1** (2.38 g, 10.0 mmol), 5-(2-bromo-1-ethylidene)-10,11-dihydro-5*H*-dibenzo[*a,d*]cycloheptene[47] (2.75 g, 10.0 mmol), and potassium carbonate (5.14 g, 30.0 mmol) in dimethylformamide (30 mL) was heated at 100 °C for 20 h. The reaction mixture was diluted with benzene (80 mL), washed with 5% aqueous citric acid (3 × 25 mL) and with saturated $NaHCO_3$ (25 mL), dried ($MgSO_4$), and evaporated. The residue (4.88 g) was purified by column chromatography on silica gel (benzene eluent) to yield the title compound; 2.3 g (53%). ^{1}H NMR ($CDCl_3$) δ 1.15 (t, *J* = 7 Hz, 3H), 1.95 (t, *J* = 7 Hz, 3H), 2.92 (d, *J* = 7 Hz, 2H), 3.17 (bs, 4H), 3.28 (m, 1H), 3.58 (m, 1H), 3.94 (m, 1H), 4.14 (q, *J* = 7 Hz, 2H), 4.59 (bs, 2H), 6.10 (t, *J* = 7 Hz, 1H), 6.71 (m, 2H), 7.05–7.25 (m, 9H), 7.32 (m, 1H).

The following compound was made according to procedure D using 12-(2-bromoethylidene)-12*H*-dibenzo[*d,g*]-1,3-dioxocine:

Ethyl 3-(4-(2-(12*H*-Dibenzo[*d,g*]-1,3-dioxocine-12-ylidene)ethoxy)phenyl)-2-ethoxypropionate, 2g. From 12-(2-bromoethylidene)-12*H*-dibenzo[*d,g*]-1,3-dioxocine. Yield 0.97 g (62%). ^{1}H NMR ($CDCl_3$) δ 1.15 (t, *J* = 7 Hz, 3H), 1.20 (t, *J* = 7.2 Hz, 3H), 2.93 (d, *J* = 7.1 Hz, 2H), 3.33 (m, 1H), 3.58 (m, 1H), 3.95 (t, *J* = 7.2 Hz, 1H), 4.14 (q, *J* = 7.2 Hz, 2H), 4.47 (d, *J* = 6.2 Hz, 2H), 5.90 (s, 2H), 6.21 (t, *J* = 6.2 Hz, 1H), 6.73 (d, *J* = 8.2 Hz, 2H), 6.93–7.32 (m, 7H), 7.35 (m, 2H), 7.38 (m, 1H).

12-(2-Bromoethylidene)-12*H*-dibenzo[*d,g*]-1,3-dioxocine. To a solution of vinylmagnesium bromide (prepared from vinyl bromide (8.65 g, 80.0 mmol) and magnesium turnings (2.14 g, 88.0 mmol) in tetrahydrofuran (120 mL)), which was cooled to 10 °C, was added a solution of 12*H*-dibenzo[*d,g*]-1,3-dioxocin-12-one in tetrahydrofuran (30 mL) dropwise over 25 min. The reaction mixture was stirred at room temperature for 3 h and then cooled to 0 °C, keeping the temperature between 0 and 10 °C. The mixture was decomposed with a solution of ammonium chloride (10 g) in water (50 mL). The mixture was then extracted with benzene (100 mL), the organic layer separated, and the aqueous layer extracted with additional benzene (2 × 50 mL). The combined organic extracts were dried over $MgSO_4$ and evaporated in vacuo. The residue (13.7 g) was purified by column chromatography on silica gel (200 g). Benzene and benzene/ethyl acetate fractions afforded 12-vinyl-12*H*-dibenzo[*d,g*]-1,3-dioxocin-12-ol (6.0 g), mp 93–98 °C. ^{1}H NMR ($CDCl_3$) δ 2.57 (bs, 1 H), 5.08 (d, *J* = 5.0 Hz, 1H); 5.21 (d, *J* = 5.0 Hz, 1H), 5.12 (dd, *J* = 1.3 and 10.6 Hz, 1H), 5.43 (dd, *J* = 1.3 and *J* = 17.0 Hz, 1H), 6.47 (dd, *J* = 10.6 and *J* = 17.0 Hz, 1H), 7.02 (dd, *J* = 1.6 and *J* = 7.5 Hz, 2H), 7.20 (dt, *J* = 1.6 and *J* = 7.5 Hz, 2H), 7.28 (dt, *J* = 1.9 and *J* = 7.5 Hz, 2H); 7.75 (dd, *J* = 1.9 and *J* = 7.5 Hz, 2H).

To a solution of the above dioxocinol (2.2 g, 8.6 mmol) in dichloromethane (95 mL), was added trimethylbromosilane (1.50 g, 9.8 mmol) in several portions through a septum inlet under an atmosphere of nitrogen. The reaction mixture was stirred at room temperature for 1.5 h and poured into saturated sodium hydrogen carbonate solution (30 mL). The organic layer was separated, washed with water (2 × 20 mL) and brine (20 mL), and dried over $MgSO_4$. After evaporation of the solvent in vacuo, the crude oily 12-(2-bromoethylidene)-12*H*-dibenzo[*d,g*]-1,3-dioxocine (2.5 g, 91%) was used in the above step without further purification. ^{1}H NMR ($CDCl_3$) δ 3.89 (d, *J* = 8.5 Hz, 2H), 5.89 (s, 2H), 6.22 (t, *J* = 8.5 Hz, 1H), 7.14 (m, 8H).

3-(4-[2-(Dibenzo[*b,f*]-1,4-thiazepin-11-ylamino)ethoxy]-phenyl)-2-ethoxypropanoic Acid, 3b. Ethyl 3-(4-[2-(dibenzo[*b,f*]-1,4-thiazepin-11-ylamino)ethoxy]phenyl)-2-ethoxypropionate (1.6 g, 3.26 mmol) was dissolved in ethanol (30 mL), and 20% sodium hydroxide (3 mL) was added. After 6 days the ethanol was evaporated in vacuo, water (50 mL) and acetic acid (3 mL) were added, and the product was filtered off and dried, yielding 1.4 g (87%) of the title compound as the hydrate. ^{1}H NMR (DMSO-d_6) δ 1.03 (t, *J* = 7.2 Hz, 3H), 2.70–2.82 (m, 1H), 2.91 (m, 1H), 3.25 (m, 1H), 3.54 (m, 1H), 3.78 (bs, 2H), 3.88 (dd, *J* = 7.6 and 4.2 Hz, 1H), 4.26 (t, *J* = 4.9 Hz, 2H), 6.82–7.15 (m, 4H), 7.10–7.25 (m, 3H), 7.3–7.6 (m, 6H).

General Procedure for Hydrolysis of the Ester to the Final Acid. 3-(4-(2-(10,11-Dihydro-dibenzo[*b,f*]azepin-5-yl)ethoxy)phenyl)-2-ethoxypropionic Acid, 3m. A solution of ethyl 3-(4-(2-(10,11-dihydro-dibenzo[*b,f*]azepin-5-yl)ethoxy)-phenyl)-2-ethoxypropionate (191 mg, 0.42 mmol) in ethanol (13 mL) and aqueous 1 N sodium hydroxide (4.5 mL) was stirred at 90 °C for 1 h. The reaction mixture was evaporated and the residue dissolved in water (7 mL). The aqueous phase was extracted with ethyl acetate (2 × 50 mL) after acidification with 1 N HCl (7.5 mL). The combined organic phases were dried, evaporated, and purified by column chromatography, using dichloromethane:methanol (9:1) as eluent, to give the title compound in 176 mg (97%) yield. ^{1}H NMR ($CDCl_3$) δ 1.1 (t, *J* = 7 Hz, 3H), 2.72–3.06 (m, 2H), 3.17 (s, 4H), 3.35 (m, 1H), 3.55 (m, 1H), 3.94–4.05 (m, 3H), 4.15 (t, *J* = 7 Hz, 2H), 6.69 (d, *J* = 8 Hz, 2H), 6.85–6.95 (m, 2H), 7.03–7.15 (m, 8H), 8.5–9.0 (bs, 1H). MS 431 (M^+), 222, 208 (100%), 193, 165, 91.

The following compounds were made as described above using the appropriate starting material, **2**.

3-(4-(3-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)propoxy)phenyl)-2-ethoxypropionic Acid, 3c, L-Lysine Salt. The resulting residue (free acid; 1.1 g, 78%) was dissolved in ethanol and treated with L-lysine monohydrate (0.41 g), and the ethanol was evaporated. The residue was triturated with diethyl ether, and the crystalline product was collected by filtration and air-dried to give the title salt as the dihydrate. Yield 1.45 g, mp 148–150 °C. ^{1}H NMR (DMSO) δ 1.03 (t, *J* = 7 Hz, 3H), 1.66 (m, 6H), 2.51 (m, 2H), 2.70–2,95 (m, 4H), 3.07 (bs, 4H), 3.31–3.59 (m, 2H), 3.76 (m, 1H), 4.02 (t, *J* = 6 Hz, 2H), 5.91 (t, *J* = 7 Hz, 1H), 6.26 (bs, 8H), 6.75 (dd, *J* = 8 and 2H), 7.00–7.35 (m, 10H).

3-(4-[2-(10,11-Dihydro-dibenzo[*a,d*]cyclohepten-5-ylidene)ethoxy]phenyl)-2-ethoxypropionic Acid, 3d. The residue was crystallized from a mixture of toluene (8 mL) and *n*-heptane (8 mL) to give the title compound in 1.60 g (74%) yield; mp 147–150 °C. ^{1}H NMR ($CDCl_3$) δ 1.15 (t, *J* = 7 Hz, 3H), 2.99 (m, 2H), 3.17 (bs, 4H), 3.42–3.58 (m, 2H), 4.01 (dd, *J* = 8 and 4 Hz, 1H), 4.59 (bd, 2H), 6.11 (t, *J* = 7 Hz, 1H), 6.72 (m, 2H), 7.02–7.35 (m, 10H).

3-(4-(3-(6*H*-Dibenzo[*b,e*]oxepin-11-ylidene)propoxy)-phenyl)-2-ethoxypropionic Acid, 3e. Isolated as an inseparable 4:1 mixture of *E* and *Z* double-bond isomers, as a pale yellow glass; 186 mg (80%). ^{1}H NMR ($CDCl_3$) δ 1.16 (t, *J* = 7 Hz, 3H), 2.65 (q, *J* = 7 Hz, 1.6H, *E* isomer), 2.90 (q, *J* = 7 Hz, 0.4 H, *Z* isomer), 2.93 (dd, *J* = 14 and 9 Hz, 1H), 3.05 (dd, *J* = 14 and 5 Hz, 1H), 3.32–3.70 (m, 2H), 3.94–4.10 (m, 3H), 4.5–5.7 (very broad m, 2H), 5.80 (t, *J* = 7 Hz, 0.25H, *Z* isomer), 6.12 (t, *J* = 7 Hz, 0.75H, *E* isomer), 6.7–6.95 (m, 4H), 7.05–7.20 (m, 2H), 7.20–7.40 (m, 6H). MS: 444 (M^+), 341, 326, 235 (100%), 221, 195, 107, 91.

2-Ethoxy-3-(4-(2-(11*H*-5-oxa-10-thia-dibenzo[*a,d*]cyclohepten-11-yl)ethoxy)phenyl)propionic Acid, 3f, L-Lysine Salt. The resulting residue (3.8 g, 88%) was dissolved in ethanol and treated with L-lysine (1.25 g), the solvent evaporated, and the residue triturated with diethyl ether. The resulting crystalline product was collected by filtration and air-dried to give the title salt: 4.35 g; mp 153.5–154.5 °C. ^{1}H NMR (DMSO-d_6) δ 1.00 (t, *J* = 7 Hz, 3H), 1.2–2.0 (m, 6H), 2.4–3.0 (m, 6H), 3.15 (m, 1H); 3.43 (m, 1H), 3.56 (m, 1H), 3.66 (bs, 1H), 4.00 (bs, 1H), 4.13 (bs, 1H), 4.90 (t, *J* = 6.7 Hz, 1H), 6.82 (d, *J* = 7.9 Hz, 2H), 7.00–7.50 (m, 10H), 7.82 (bs, 5H).

3-(4-(2-(12*H*-Dibenzo[*d,g*]-1,3-dioxocine-12-ylidene)-ethoxy)phenyl)-2-ethoxypropionic Acid, 3g, L-Lysine Salt. Yield 0.79 g (83%). This acid (0.76 g, 1.6 mmol) was dissolved in acetone (30 mL), L-lysine (0.234 g, 1.6 mmol) in water (3 mL) was added, and the mixture was stirred at room temperature for 2 h. The solution was filtered and evaporated, and the residue was stirred with a mixture of Et_2O (20 mL) and acetone (20 mL) overnight. The resulting solid was collected by filtration, washed with Et_2O (2 × 30 mL), and dried to give the title compound as a partial hydrate: 0.90 g (93.5%); mp 162–168 °C. ^{1}H NMR (DMSO-d_6) δ 1.04 (t, *J* = 6.8 Hz, 3H), 1.38–1.89 (m, 6H), 2.75 (m, 3H), 2.90 (dd, *J* =

14.4 and 4.3 Hz, 1H), 3.27 (m, 3H), 3.58 (m, 1H), 3.75 (m, 1H) 4.49 (d, $J = 6.9$ Hz, 2H), 5.89 (bs, 10H), 6.20 (t, $J = 6.3$ Hz, 1H), 6.75 (d, $J = 7.7$ Hz, 2H), 6.91–7.52 (m, 10H).

2-Ethoxy-3-(4-[2-(9*H*-xanthen-9-yl)ethoxy]phenyl)propionic Acid, 3h. Gave the title compound as an oil. Yield 1.3 g (77%). ^{1}H NMR ($CDCl_3$) δ 1.17 (t, $J = 6.4$ Hz, 3H), 2.11 (q, $J = 6.4$ Hz, 2H), 2.80–3.20 (m, 2H), 3.40–3.70 (m, 3H), 3.82 (t, $J = 6.8$ Hz, 2H), 4.04 (dd, $J = 4.3$ and 7.6 Hz, 1H), 4.28 (t, $J = 6.8$ Hz, 1H), 6.77 (d, $J = 8.6$ Hz, 2H), 7.01–7.24 (m, 10H).

2-Ethoxy-3-(4-[2-(9*H*-fluoren-9-yl)ethoxy]phenyl)propionic Acid, 3j. Yield 170 mg (95%). ^{1}H NMR $CDCl_3$) δ 1.17 (t, $J = 7$ Hz, 3H), 2.46 (q, $J = 7$ Hz, 2H), 2.93 (dd, $J = 16$ and 7 Hz, 1H), 3.04 (dd, $J = 16$ and 5 Hz, 1H), 3.38–3.50 (m, 1H), 3.50–3.65 (m, 1H), 3.90 (t, $J = 7$ Hz, 2H), 4.04 (dd, $J = 7$ and 5 Hz, 1H), 4.23 (t, $J = 7$ Hz, 1H), 6.74 (d, $J = 8$ Hz, 2H), 7.11 (d, $J = 8$ Hz, 2H), 7.25–7.42 (m, 4H), 7.52 (d, $J = 8$ Hz, 2H), 7.75 (d, $J = 8$ Hz, 2H). MS 402 (M^+), 299, 193, 178, 165 (100%), 107.

3-(4-Dibenzo[*d,g*]dioxazocin-12-yl)-1-propoxy)phenyl-2-ethoxypropionic Acid, 3k. Yield 1.92 g (79%). ^{1}H NMR ($CDCl_3$) δ 1.15 (t, $J = 7.2$ Hz, 3H), 1.95 (q, $J = 5.7$ Hz, 2H), 3.1–2.85 (m, 2H), 3.6–3.4 (m, 2H), 3.81 (t, $J = 5.7$ Hz, 2H), 3.97 (t, $J = 5.7$ Hz, 2H), 4.00 (m, 1H), 5.71 (s, 2H), 6.75 (dt, $J = 8.8$ Hz, 2H), 7.20–6.95 (m, 10H).

3-(4-(2-(10,11-Dihydro-dibenzo[*b,f*]azepin-5-yl)propoxy)-phenyl)-2-ethoxypropionic Acid, 3l. Yield 1.65 g (98%). ^{1}H NMR (DMSO) δ 1.17 (t, $J = 7$ Hz, 3H), 2.04 (q, $J = 7$ Hz, 2H), 2.91 (dd, $J = 7.3$ and 14.3 Hz, 1H), 3.08 (dd, $J = 4.3$ and 14.3 Hz, 1H), 3.15 (s, 4H), 3.61–3.42 (m, 2H), 3.92 (t, $J = 7$ Hz, 2H), 3.97 (t, $J = 7$ Hz, 2H), 4.04 (dd, $J = 4.3$ and 7.3 Hz, 1H), 6.73 (m, 2H), 6.92 (m, 2H), 7.05–7.15 (m, 8H).

3-(4-(2-(Dibenzo[*b,f*]azepin-5-yl)ethoxy)phenyl)-2-ethoxypropionic Acid, 3n. Yield 151 mg (93%). ^{1}H NMR ($CDCl_3$) δ 1.12 (t, $J = 7$ Hz, 3H), 2.84–3.05 (m, 2H), 3.28–3.40 (m, 1H), 3.50–3.62 (m, 1H), 3.93–4.18 (m, 5H), 6.75 (m, 4H), 6.95–7.78 (m, 10H), 8.5–9.0 (bs, 1H). MS 429 (M^+), 220, 207, 206 (100%), 178, 165, 128, 91.

3-(4-(2-(β-Carbolin-9-yl)ethoxy)phenyl)-2-ethoxypropionic Acid, 3o. Yield 50 mg (30%). ^{1}H NMR ($CDCl_3$) δ 1.20 (t, $J = 7$ Hz, 3H), 3.03 (d, $J = 7$ Hz, 2H), 3.38–3.52 (m, 1H), 3.62–3.76 (m, 1H), 4.10 (t, $J = 7$ Hz, 1H), 4.37 (t, $J = 7$ Hz, 2H), 4.70 (t, $J = 7$ Hz, 2H), 6.60 (d, $J = 8$ Hz, 2H), 7.17 (d, $J = 8$ Hz, 2H), 7.35–7.73 (m, 3H), 8.08 (d, $J = 7$ Hz, 1H), 8.19 (d, $J = 8$ Hz, 1H), 8.41 (d, $J = 7$ Hz, 1H), 8.67 (s, 1H), 8.8–9.3 (bs, 1H).

(*S*)-3-(4-(2-(β-Carbolin-9-yl)ethoxy)phenyl)-2-ethoxypropionic Acid, 3p. Yield 850 mg (70%). ^{1}H NMR ($CDCl_3$) δ 1.20 (t, $J = 7$ Hz, 3H), 3.03 (d, $J = 7$ Hz, 2H), 3.38–3.52 (m, 1H), 3.62–3.76 (m, 1H), 4.10 (t, $J = 7$ Hz, 1H), 4.37 (t, $J = 7$ Hz, 2H), 4.70 (t, $J = 7$ Hz, 2H), 6.60 (d, $J = 8$ Hz, 2H), 7.17 (d, $J = 8$ Hz, 2H), 7.35–7.73 (m, 3H), 8.08 (d, $J = 7$ Hz, 1H), 8.19 (d, $J = 8$ Hz, 1H), 8.41 (d, $J = 7$ Hz, 1H), 8.67 (s, 1H), 8.8–9.3 (bs, 1H). 96.1% ee.

(*S*)-3-(4-(2-Carbazol-9-yl-ethoxy)phenyl)-2-ethoxypropionic Acid, 3q. Yield 7.0 g (100%). ^{1}H NMR ($CDCl_3$) δ 1.15 (t, $J = 7$ Hz, 3H), 2.85–3.06 (m, 2H), 3.35 (m, 2H), 3.55 (m, 2H), 3.98 (m, 1H), 4.30 (t, $J = 7$ Hz, 2H), 4.70 (t, $J = 7$ Hz, 2H), 6.72 (d, $J = 8$ Hz, 2H), 7.08 (d, $J = 8$ Hz, 2H), 7.25 (m, 2H), 7.47 (m, 4H), 8.07 (m, 2H). 98.6% ee.

(*S*)-3-(4-(2-(3-Bromo-carbazol-9-yl)ethoxy)phenyl)-2-ethoxypropionic Acid, 3r. Yield 290 mg (87%). ^{1}H NMR (MeOH) Na-salt δ 1.07 (t, $J = 7$ Hz, 3H), 2.74 (m, 1H), 2.88 (m, 1H), 3.20 (m, 1H), 3.57 (m, 1H), 3.74 (m, 1H), 4.35 (t, $J = 7$ Hz, 2H), 4.75 (t, $J = 7$ Hz, 2H), 6.67 (d, $J = 8$ Hz, 2H), 7.12 (d, $J = 8$ Hz, 2H), 7.22 (t, $J = 8$ Hz, 1H), 7.50 (m, 3H), 7.60 (d, $J = 8$ Hz, 1H), 8.06 (d, $J = 8$ Hz, 1H), 8.19 (s, 1H). 95.9% ee.

(*S*)-3-(4-(2-(3,6-Dibromo-carbazol-9-yl)ethoxy)phenyl)-2-ethoxypropionic Acid, 3s. Yield 75 mg (39%). ^{1}H NMR ($CDCl_3$) δ 1.17 (t, $J = 7$ Hz, 3H), 2.93 (m, 1H), 3.06 (m, 1H), 3.63–3.35 (m, 2H), 4.03 (m, 1H), 4.32 (t, $J = 7$ Hz, 2H), 4.67 (t, $J = 7$ Hz, 2H), 6.68 (d, $J = 8$ Hz, 2H), 7.07 (d, $J = 8$ Hz, 2H), 7.38 (d, $J = 8$ Hz, 2H), 7.58 (d, $J = 8$ Hz, 2H), 8.15 (s, 2H). 94.3% ee.

2-Ethoxy-3-[4-(2-fluoren-9-ylidene-ethoxy)phenyl]propionic Acid, 3i. Lithium hydroxide (1 M, 1.0 mL, 1.0 mmol) was added to a suspension of ethyl 2-ethoxy-3-[4-(2-fluoren-9-ylidene-ethoxy)phenyl]propionate (214 mg, 0.5 mmol) in ethanol (5 mL), and the resulting mixture was heated to gentle reflux for 30 min. The cooled mixture was partitioned between water (30 mL) and dichloromethane (20 mL) and acidified to pH 1 by adding 1 N HCl (3 mL), and the organic phase was collected. The aqueous phase was further extracted with dichloromethane (3 × 20 mL), and the combined organics were washed with brine, dried ($MgSO_4$), and evaporated to give a yellow gum. The product was purified by column chromatography on silica gel (3% methanol in dichloromethane eluent) to give 2-ethoxy-3-[4-(2-fluoren-9-ylidene-ethoxy)phenyl]propionic acid, as a yellow solid; 0.104 g (51%). ^{1}H NMR ($CDCl_3$) δ 1.17 (t, $J = 7$ Hz, 3H), 2.98 (dd, $J = 14$ and 7 Hz, 1H), 3.10 (dd, $J = 14$ and 4 Hz, 1H), 3.40–3.70 (m, 2H), 4.06 (dd, $J = 7$ and 4 Hz, 1H), 5.33 (d, $J = 6$ Hz, 2H), 6.87 (t, $J = 6$ Hz, 1H), 6.98 (d, $J = 8$ Hz, 2H), 7.20 (d, $J = 8$ Hz, 2H), 7.20–7.47 (m, 4H), 7.55–7.80 (m, 4H). MS: 400 (M^+), 435, 297, 235, 209, 191 (100%), 165.

General Procedure for Crystallization as Arginine Salt. (*S*)-3-(4-(2-Carbazol-9-yl-ethoxy)phenyl)-2-ethoxypropionic Acid, 3q, L-Arginine. A solution of L-arginine (2.9 g, 16.67 mmol) in water (10 mL) was dropwise added to a 60 °C warm stirring solution of (*S*)-3-(4-(2-carbazol-9-yl-ethoxy)phenyl)-2-ethoxypropionic acid, **3q** (7.0 g, 16.6 mmol), in ethanol (250 mL). The mixture was stirred at room temperature overnight, and the crystals were collected by filtration and dried. Yield 9.5 g (99%). ^{1}H NMR (CD_3OD) δ 1.07 (t, $J = 7$ Hz, 3H), 1.60–1.73 (m, 2H), 1.78–1.90 (m, 2H), 2.85 (dd, $J = 8$ and 16 Hz, 1H), 2.90 (dd, $J = 5$ and 16 Hz), 3.10–3.30 (m, 3H), 3.50–3.63 (m, 2H), 3.85 (q, $J = 4$ Hz, 1H), 4.34 (t, $J = 4$ Hz, 2H), 4.73 (t, $J = 4$ Hz, 2H), 6.67 (d, $J = 8$ Hz, 2H), 7.09 (d, $J = 8$ Hz, 2H), 7.19 (t, $J = 7$ Hz; 2H), 7.45 (t, $J = 7$ Hz, 2H), 7.57 (d, $J = 7$ Hz, 2H), 8.06 (d, $J = 7$ Hz, 2H).

In Vitro Transactivation Assays. Cell Culture and Transfection. HEK293 cells were grown in DMEM + 10% FCS. Cells were seeded in 96-well plates the day before transfection to give a confluency of 50–80% at transfection. A total of 0.8 μg of DNA containing 0.64 μg of pM1α/γLBD, 0.1 μg of pCMVβGal, 0.08 μg of pGL2(Gal4)$_5$, and 0.02 μg of pADVANTAGE was transfected per well using FuGene transfection reagent according to the manufacturers instructions (Roche). Cells were allowed to express protein for 48 h followed by addition of compound.

Plasmids. Human PPARα and PPARγ was obtained by PCR amplification using cDNA synthesized by reverse transcription of mRNA from liver and adipose tissue, respectively. Amplified cDNAs were cloned into pCR2.1 and sequenced. The ligand binding domain (LBD) of each PPAR isoform was generated by PCR (PPARα: aa 167 – C-terminus; PPARγ: aa 165 – C-terminus) and fused to the DNA binding domain (DBD) of the yeast transcription factor GAL4 by subcloning fragments in frame into the vector pM1[48] generating the plasmids pM1αLBD and pM1γLBD. Ensuing fusions were verified by sequencing. The reporter was constructed by inserting an oligonucleotide encoding five repeats of the GAL4 recognition sequence (5 × CGGAGTACTGTCCTCCG(AG))[49] into the vector pGL2 promotor (Promega) generating the plasmid pGL2(GAL4)$_5$. pCMVβGal was purchased from Clontech and pADVANTAGE was purchased from Promega.

Luciferase Assay. Medium including test compound was aspirated, and 100 μL PBS including 1 mM Mg^{2+} and Ca^{2+} was added to each well. The luciferase assay was performed using the LucLite kit according to the manufacturers instructions (Packard Instruments). Light emission was quantified by counting SPC mode on a Packard Instruments top-counter. To measure β-galactosidase activity, 25 μL of supernatant from each transfection lysate was transferred to a new microplate. β-Galactosidase assays were performed in the microwell plates using a kit from Promega and read in a Labsystems Ascent Multiscan reader. The β-galactosidase data were used

to normalize (transfection efficiency, cell growth, etc.) the luciferase data.

Compounds. All compounds were dissolved in DMSO and diluted 1:1000 upon addition to the cells. Compounds were tested in quadruple in five concentrations ranging form 0.01 to 30 μM. Cells were treated with compound for 24 h followed by luciferase assay. Each compound was tested in three separate experiments. EC_{50} values were calculated via nonlinear regression using GraphPad PRISM 3.02 (GraphPad Software, San Diego, CA). The results were expressed as means $\pm$ SD.

In Vivo Models. C57BL/KsBom-db/db and lean db/+ male mice, 14 weeks old, were purchased from BOMMICE, Bomholtgård Breeding & Research centre A/S, Ry, DK. The mice were housed in groups of six individuals in a room controlled for temperature (20.0 $\pm$ 0.5 °C) and 12/12 h light/dark cycle (lights on at 6.00 am). The mice had free access to normal chow and water.

Compounds were dosed as suspensions in 0.2% CMC + 0.4% Tween-80 in saline. Fresh suspensions were made for 7 days dosing and kept at +4 °C. The mice ($n = 6$ per dose) were dosed orally by gavage daily at 7.30 am from day 1 to 10. The dose volume was 10 mL/kg.

Samples and Analyses. A total of 5 μL of A total of nonfasted full blood was drawn from the tail vein for measuring baseline glucose. After 7 days of treatment, blood was drawn from the orbital plexus in nonfasted animals. Blood was collected in EDTA tubes and centrifuged at 4000*g* for 10 min at 4 °C. Plasma was analyzed for triglycerides on a COBAS-Mirra, and full blood glucose was measured on a EBIO-plus. Insulin was measured using ELISA.

On day 9 of treatment, an oral glucose tolerance test (OGTT) was performed on overnight fasted animals. A 5 μL full blood baseline sample was drawn from the tail vein before glucose dosing (3 g/kg). After administration of the glucose, 5 μL full blood samples were drawn at 30, 60, and 120 min from the tail vein. All samples from OGTT were analyzed for glucose on an EBIO-plus.

Six weeks old male Sprague–Dawley rats (Charles River, Germany) were fed on a high cholesterol diet ad libitum (1.25% cholesterol, 0.5% cholic acid; Research Diets Inc. C13002) for 10 days. From day 7 to day 10, the animals ($n = 6$ per dose) were dosed orally by gavage at 7.30 a.m. Test compounds were suspended in vehicle (0.2% CMC + 0.4% Tween 80 in sterile water) and administered in a volume of 2 mL/kg.

Two hours after last dosing on day 10, 2 mL of nonfasting orbital vein plexus blood was collected and allowed to coagulate for 30 min on wet ice. Serum was separated by centrifugation (4000*g* for 10 min at 4 °C) and stored at −70 °C until analyzed for triglycerides and cholesterol on a COBAS-Mirra.

Statistics. ED_{50} values were calculated via nonlinear regression using GraphPad PRISM 3.02 (GraphPad Software, San Diego, CA). The results were expressed as means $\pm$ SEM. Differences between two groups were evaluated by one way ANOVA and Dunett's multiple comparison test $^*P < 0.05$, $^{**}P < 0.01$. P values less than 0.05 were considered significant.

Percent reduction was calculated using the equation

$$((C_v - C_t)/C_v) \times 100$$

and percent normalization using the equation

$$((C_v - C_t)/(C_v - C_l)) \times 100$$

where C_v was the plasma concentration in the vehicle treated group, C_t the plasma concentration in the compound treated group, and C_l the concentration in the lean vehicle treated group.

Crystallography. Ligand binding domain (LBD, amino acids C_{165} − Stop) PPARγ was expressed, purified, and crystallized according to Ebdrup et al., 2002.[50] In short, LBD-PPARγ fused to glutathione S-transferase was expressed in *Escherichia coli*, the protein was purified by a GSH-Sepharose column and thereafter cleaved, and the GST was removed. The LBD-PPARγ protein was crystallized in 0.8 M sodium citrate and 0.15 M Tris, pH 8.0, at a protein concentration of 5 mg/mL. The crystal space group and cell parameters obtained are found in Table X+1, Supporting Information. A crystal storage solution containing 1.0 M sodium citrate and 0.15 M Tris, pH 8.0, was prepared. Compound **3q** was dissolved in 20 μL storage solution after which crystals were transferred and soaked for 24 h. Crystals were then transferred over during 10 to 30 s to a cryo-protectant containing the storage solution mixed with glycerol to a concentration of 20% (v/v). The crystals were thereafter flash-frozen in a nitrogen gas-stream cooled to 100 K, their diffraction properties tested and then stored in liquid nitrogen for subsequent data collection. Crystallographic data were collected at beamline I711, the MAX-laboratory, Sweden,[51] using a mar345 imaging plate detector system, and data sets were evaluated by the Xds program package.[52] The structure was subsequently refined by the Cnx program system,[53] in the starting run making use of the PPARγ coordinates generated by Ebdrup et al. (2002),[50] which in turn was based on the coordinates 1PRG of the Protein Data Bank deposited by Nolte et al.[29] Introduction of **3q** and corrections to the model according to electron density maps were made with use of the Quanta program.[54] The program Xplo2d[55] was used for creation of ligand Parameter and Topology files used by the Cnx program. For data collection, refinement, and model statistics, see Supporting Information Table X+1. The coordinates of the **3q**/PPARγ structure have been deposited in the Brookhaven Protein Data Bank, ID 1KNU.

Modeling. The Grid calculations were performed with Grid ver.18[56–59] with DPRO equal to 4, DWAT equal to 80, and EMAX equal to 5. The calculations were performed with 2 planes per ångstrom. All calculations on the complex with **3q** were performed using the structure based on soaked crystals. The atoms in the ligand were treated as HETATM.

Molecular mechanics calculations were performed with the MMFF force field[34–37] with water as solvation model[60] in MacroModel ver. 7.0.[38,39] The Monte Carlo searches were performed with a systematic pseudo Monte Carlo search.[61]

Pharmacokinetics. The compounds were dosed po and iv to male SD rats. The compounds were dissolved in 5% ethanol, 10% HPCD, and phosphate buffer pH 7.5–8.0. Blood samples were collected in EDTA tubes. Each data point represents one animal.

Plasma samples were analyzed by high turbulence liquid chromatography (HTLC) combined with tandem mass spectrometry (MS/MS).

Sample preparation included dilution (5% methanol, 1:1), centrifugation (14500*g* for 15 min), and aliquotation of minimum 100 μL to 96-well plates. HTLC was performed using a 2300 HTLC System (Cohesive Technologies, Franklin, MA) consisting of an isocratic pump for sample cleanup and flush of liquid lines, a binary pump for elution of retained analytes, and a valve switching module with two Rheodyne six-port valves. The 2300 HTLC system was operated in single column mode. The autosampler was a CTC HTS PAL (CTC Analytics, Zingen, Switzerland). Injection volumes were from 10 to 50 μL. The mass spectrometer was a Sciex API3000 (MDS Sciex, Toronto, Canada) equipped with a TurboIonSpray and operated in MRM mode.

Chiral Analysis. The enantiomeric purity of (*S*)-ethyl 2-ethoxy-3-(4-hydroxyphenyl)propionate (**1**), **3p**, **3q**, **3r**, and **3s** were determined by a chiral capillary electrophoresis (CE) analytical system developed for the purpose. The CE analyses were performed on a HP^{3D}CE capillary electrophoresis instrument (Agilent, Waldborn, Germany) equipped with an auto sampler, a capillary cartridge, a high-voltage power supply, a diode array detector, electrodes, and a hydrostatic injection system. The electrophoretic data system was the HP Chemstation software, and the data were collected with a frequency of 10 Hz. The CE separations were carried out with untreated fused-silica capillaries from Agilent with the following dimensions: 48.5 cm total length with 40.0 cm effective length, 50 μm inner diameter, and extended light path with an inner

diameter of 150 μm at the detector window. The electrolyte was prepared by dissolving 3.0% (w/v) sulfobuthyl ether-β-cyclodextrin (Advasep 4, Cydex, Inc., Overland Park, KS) and 0.50% (w/v) dimethyl-β-cyclodextrin (Agilent, Waldborn, Germany) both in 50 mM borate buffer pH 9.3 (Agilent) followed by filtering through a 0.45 μm polypropylene filter. To this solution was added 5% (v/v) acetonitrile to give the final electrolyte. The electrophoresis was carried out in normal polarity mode.

The electrophoretic conditions were as follows: voltage, 14 kV; current, 60 μA; capillary temperature controlled at 30 °C; injection was 25 mbar for 4.0 s; detection, UV at 231 nm with reference of 350 nm. The sample concentration was 2.5 mg/mL in 10/90 acetonitrile/5 mM borate buffer pH 9.3. The capillary was conditioned with 0.1 N NaOH for 20 min daily and flushed with electrolyte for 1.5 min between each run.

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Supporting Information Available: Table of hydrogen bonds and van der Waals interactions between **3q** and the amino acids in the PPARγ receptor protein (Table X). Table for data collection, refinement, and model statistics (Table X+1). Illustration of a capillary electrophoresis chromatogram exemplified by **3p**. This material is available free of charge via the Internet at http://pubs.asc.org.

References

(1) Porte, D., Jr.; Schwartz, M. W. Diabetes complications: Why is Glucose Potentially Toxic? *Science* **1996**, *27*, 699–700.
(2) Staels, B.; Dallongeville, J.; Auwerx, J.; Schoonjans, K.; Leitersdorf, E.; Fruchart, J.-C. Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation* **1998**, *98*, 2088–2093.
(3) Oliver, M. F.; Heady, J. A.; Morris, J. N.; Cooper, J. WHO cooperative trial on the primary prevention of ischemic heart disease with clofibrate to lower serum cholesterol: final mortality follow-up. *Lancet* **1984**, *2*, 600–604.
(4) Ericsson, C. G.; Nilsson, J.; Grip, L.; Svane, B.; Hamsten, A. Effect of bezafibrate treatment over five years on coronary plaques causing 20% to 50% diameter narrowing (the bezafibrate coronary atherosclerosis intervention trial [BECAIT]). *Am. J. Cardiol.* **1997**, *80*, 1125–1129.
(5) Rutolo, G.; Ericsson, C.-G.; Tettamanti, C.; Karpe, F.; Grip, L.; Svane, B.; Nilsson, J.; De Faire, U.; Hamsten, A. Treatment effects on serum lipoprotein lipids, apolipoproteins and low-density lipoprotein particle size and relationships of lipoprotein variables to progression of coronary artery disease in the bezafibrate coronary atherosclerosis intervention trial (BECAIT). *J. Am. Coll. Cardiol.* **1998**, *32*, 1648–1656.
(6) Rubins, H. B.; Robins, S. J.; Collins, D.; Fye, C. L.; Anderson, J. W.; Elam, M. B.; Faas, F. H.; Linares, E.; Schaefer, E. J.; Schectman, G.; Wilt, T. J.; Wittes, J. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. *N. Engl. J. Med.* **1999**, *341*, 410–418.
(7) Steiner, G.; Hamsten, A.; Hosking, J.; Stewart, D.; McLaughlin, P.; Gladstone, P.; Sole, M.; Syvanne, M. Effect of fenofibrate on progression of coronary-artery disease in type 2 diabetes: the Diabetes Atherosclerosis Intervention Study, a randomised study. *Lancet* **2001**, *357*, 905–910.
(8) Kobayashi, M.; Shigeta, Y.; Hirata, Y.; Omori, Y.; Sakamoto, N.; Nambu, S.; Baba, S. Improvement of glucose tolerance in NIDDM by clofibrate. Randomized double-blind study. *Diabetes Care* **1988**, *11*, 495–499.
(9) Jones, I. R.; Swai, A.; Taylor, R.; Miller, M.; Laker, M. F.; Alberti, K. G. Lowering of plasma glucose concentrations with bezafibrate in patients with moderately controlled NIDDM. *Diabetes Care* **1990**, *13*, 855–863.
(10) Inoue, I.; Takahashi, K.; Katayama, S.; Akabane, S.; Negishi, K.; Suzuki, M.; Ishii, J.; Kawazu, S. Improvement of glucose tolerance by bezafibrate in nonobese patients with hyperlipidemia and impaired glucose tolerance. *Diabetes Res. Clin. Pract.* **1994**, *25*, 199–205.
(11) Vazquez, M.; Merlos, M.; Adzet, T.; Laguna Juan, C. Decreased susceptibility to copper-induced oxidation of rat-lipoproteins after fibrate treatment: influence of fatty acid composition. *Br. J. Pharmacol.* **1996**, *117*, 1155–1162.
(12) Aronoff, S.; Rosenblatt, S.; Braithwaite, S.; Egan, J. W.; Mathisen, A. L.; Schneider, R. L. Pioglitazone Hydrochloride Monotherapy Improves Glycemic Control in the Treatment of Patients With Type 2 Diabetes. *Diabetes Care* **2000**, *23*, 1605–1611.
(13) Mathisen, A. L.; Brockley, M. R. Relationship Between HbA1c and Weight in the Treatment of Patients with Type 2 Diabetes. *Diabetes* **2000**, *49* (Suppl. 1), A117.
(14) Willson, T. M.; Cobb, J. E.; Cowan, D. J.; Wiethe, R. W.; Correa, I. D.; Prakash, S. R.; Beck, K. D.; Moore, L. B.; Kliever, S. A.; Lehmann, J. M. The Structure–Activity Relationship between Peroxisome Proliferator-Activated Receptor γ Agonism and the Antihyperglycemic Activity of Thiazolidinediones. *J. Med. Chem.* **1996**, *39*, 665–668.
(15) Brown, P. J.; Winegar, D. A.; Plunket, K. D.; Moore, L. B.; Lewis, M. C.; Wilson, J. G.; Sundseth, S. S.; Koble, C. S.; Wu, Z.; Chapman, J. M.; Lehmann, J. M.; Kliever, S. A.; Willson, T. M. A Ureido-Thioisobutyric Acid (GW9578) Is a Subtype-Selective PPARα Agonist with Potent Lipid-Lowering Activity. *J. Med. Chem.* **1999**, *42*, 3785–3788.
(16) Henke, B. R.; Blanchard, S. G.; Brackeen, M. F.; Brown, K. K.; Cobb, J. E.; Collins, J. L.; Harrington, W. W.; Hashim, M. A.; Hull-Ryde, E. A.; Kaldor, I.; Kliewer, S. A.; Lake, D. H.; Lesnitzer, L. M.; Lehmann, J. M.; Lenhard, J. M.; Orband-Miller, L. A.; Miller, J. F.; Mook, R. A., Jr.; Noble, S. A.; Oliver, W., Jr.; Parks, D. J.; Plunket, K. D.; Szewczyk, J. R.; Willson, T. M. *N*-(2-Benzoylphenyl)-L-tyrosine PPARγ Agonists. 1. Discovery of a Novel Series of Potent Antihyperglycemic and Antihyperlipidemic Agents. *J. Med. Chem.* **1998**, *41*, 5020–5036.
(17) Collins, J. L.; Blanchard, S. G.; Boswell, G. E.; Charifson, P. S.; Cobb, J. E.; Henke, B. R.; Hull-Ryde, E. A.; Kazmierski, W. M.; Lake, D. H.; Leesnitzer, L. M.; Lehmann, J.; Lenhard, J. M.; Orband-Miller, L. A.; Gray-Nunez, Y.; Parks, D. J.; Plunkett, K. D.; Tong, W.-Q. *N*-(2-Benzoylphenyl)-L-tyrosine PPARγ agonists. 2. Structure–activity relationship and optimization of the phenyl alkyl ether moiety. *J. Med. Chem.* **1998**, *41*, 5037–5054.
(18) Cobb, J. E.; Blanchard, S. G.; Boswell, E. G.; Brown, K. K.; Charifson, P. S.; Cooper, J. P.; Collins, J. L.; Dezube, M.; Henke, B. R.; Hull-Ryde, E. A.; Lake, D. H.; Lenhard, J. M.; Oliver, W., Jr.; Oplinger, J.; Pentti, M.; Parks, D. J.; Plunket, K. D.; Tong, W.-Q. *N*-(2-Benzoylphenyl)-L-tyrosine PPARγ agonists. 3. Structure–activity relationship and optimization of the *N*-aryl substituent. *J. Med. Chem.* **1998**, *41*, 5055–5069.
(19) Murakami, K.; Tobe, K.; Ide, T.; Mochizuki, T.; Ohashi, M.; Akanuma, Y.; Yazaki, Y.; Kadowaki, T. A novel insulin sensitizer acts as a coligand for peroxisome proliferator-activated receptor-α (PPAR-α) and PPAR-γ: effect of PPAR-α activation on abnormal lipid metabolism in liver of Zucker fatty rats. *Diabetes* **1998**, *47*, 1841–1847.
(20) Nomura, M.; Kinoshita, S.; Satoh, H.; Maeda, T.; Murakami, K.; Tsunoda, M.; Miyachi, H.; Awano, K. (3-Substituted benzyl)-thiazolidine-2,4-diones as structurally new antihyperglycemic agents. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 533–538.
(21) (a) Brooks, D. A.; Etgen, G. J.; Rito, C. J.; Shuker, A.; Dominianni, S. J.; Warshawsky, A. M.; Ardecky, R.; Paterniti, J. R.; Tyhonas, J.; Karanewsky, D. S.; Kauffman, R. F.; Broderick, C. L.; Oldham, B. A.; Montrose-Rafizadeh, C.; Winneroski, L. L.; Faul, M. M.; McCarthy, J. R. Design and Synthesis of 2-Methyl-2-{4-[2-(5-methyl-2-aryloxazol-4-yl)ethoxy]phenoxy}propionic Acids: A New Class of Dual PPARα/γ Agonists. *J. Med. Chem.* **2001**, *44*, 2061–2064. (b) Lohray, B. B.; Lohray, V. B.; Bajji, A. C.; Kalchar, S.; Poondra, R. R.; Padakanti, S.; Chakrabati, R.; Vikramadithyan, R. K.; Misra, P.; Juluri, S.; Mamidi, N. V. S. R.; Rajagopalan, R. (−)3-[4-[2-(Phenoxazin-10-yl)ethoxy]phenyl]-2-ethoxypropanoic Acid [(−)DRF2725]: A Dual PPAR Agonist with Potent Antihyperglycemic and Lipid Modulating Activity. *J. Med. Chem.* **2001**, *44*, 2675–2678.
(22) Sohda, T.; Mizuno, K.; Kawamatsu, Y. Studies on antidiabetic agents. VI. Asymmetric transformation of (±)-5-[4-(1-methylcyclohexylmethoxy)benzyl]-2,4-thiazolidinedione (ciglitazone) with optically active 1-phenylethylamines. *Chem. Pharm. Bull.* **1984**, *32*, 4460–4465.
(23) Parks, D. J.; Tomkinson, N. C. O.; Villeneuve, M. S.; Blanchard, S. G.; Willson, T. M. Differential activity of rosiglitazone enantiomers at PPARγ. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3657–3658.
(24) Buckle, D. R.; Cantello, B. C. C.; Cawthorne, M. A.; Coyle, P. J.; Dean, D. K.; Faller, A.; Haigh, D.; Hindley, R. M.; Jefcott, L. J.; Lister, C. A.; Pinto, I. L.; Rami, H. K.; Smith, D. G.; Smith, S. A. Nonthiazolidinedione antihyperglycemic agents. 1: α-Heteroatom substituted β-phenylpropanoic acids. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2121–2126.

(25) Buckle, D. R.; Cantello, B. C. C.; Cawthorne, M. A.; Coyle, P. J.; Dean, D. K.; Faller, A.; Haigh, D.; Hindley, R. M.; Jefcott, L. J.; Lister, C. A.; Pinto, I. L.; Rami, H. K.; Smith, D. G.; Smith, S. A. Nonthiazolidinedione antihyperglycemic agents. 2: α-Carbon substituted β-phenylpropanoic acids. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2127–2130.

(26) Willson, T. M.; Brown, P. J.; Sternbach, D. D.; Henke, B. R. The PPARs: From Orphan Receptors to drug Discovery. *J. Med. Chem.* **2000**, *43*, 527–550.

(27) Hulin, B.; Newton, L. S.; Lewis, D. M.; Genereux, P. E.; Gibbs, E. M.; Clark, D. A. Hypoglycemic Activity of a Series of α-Alkylthio and α-Alkoxy Carboxylic Acids Related to Ciglitazone. *J. Med. Chem.* **1996**, *39*, 3897–3907.

(28) Haigh, D.; Birrell, H. C.; Cantello, B. C. C.; Hindley, R. M.; Ramaswamy, A.; Rami, H. K.; Stevens, N. C. Nonthiazolidinedione antihyperglycaemic agents. Part 4: Synthesis of (±)-, R-(+)- and (S)-(−)-enantiomers of 2-oxy-3-arylpropionic acids. *Tetrahedron: Asymmetry* **1999**, *10*, 1335–1351.

(29) Nolte, R. T.; Wisely, G. B.; Westin, S.; Cobb, J. E.; Lambert, M. H.; Kurokawa, R.; Rosenfeld, M. G.; Willson, T. M.; Glass, C. K.; Milburn, M. V. Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-gamma. *Nature* **1998**, *395*, 137–143.

(30) Haigh, D.; Allen, G.; Birrell, H. C.; Buckle, D. R.; Cantello, B. C. C.; Eggleston, D. S.; Haltiwanger, R. C.; Holder, J. C.; Lister, C. A.; Pinto, I. L.; Rami, H. K.; Sime, J. T.; Smith, S. A.; Sweeney, J. D. Nonthiazolidinedione Antihyperglycaemic Agents. Part 3: The Effects of Stereochemistry on the Potency of α-Methoxy-β-phenylpropionic Acids. *Bioorg. Med. Chem.* **1999**, *7*, 821–830.

(31) Loray, B. B.; Bhushan, V.; Rao, B. P.; Madhavan, G. R.; Murali, N.; Rao, K. N.; Reddy, A. K.; Reddy, P. G.; Chakrabarti, R.; Vikramadithyan, R. K.; Rajagopalan, R.; Mamidi, R. N. V. S.; Jajoo, H. K.; Subramaniam, S. Novel Euglycemic and Hyperlipidemic Agents. 1. *J. Med Chem.* **1998**, *41*, 1619–1630.

(32) Oberfield, J. L.; Collins, J. L.; Holmes, C. P.; Goreham, D. M.; Cooper, J. P.; Cobb, J. E.; Lenhard, J. M.; Hull-Ryde, E. A.; Mohr, C. P.; Blanchard, S. G.; Parks, D. J.; Moore, L. B.; Lehmann, J. M.; Plunket, K.; Miller, A. B.; Milburn, M. V.; Kliewer, S. A.; Willson, T. M. A peroxisome proliferator-activated receptor gamma ligand inhibits adipocyte differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6102–6106.

(33) Xu, H. E.; Lambert, M. H.; Montana, V. G.; Parks, D. J.; Blanchard, S. G.; Brown, P. J.; Sternbach, D. D.; Lehmann, J. M.; Wisely, G. B.; Willson, T. M.; Kliewer, S. A.; Milburn, M. V. Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Mol. Cell.* **1999**, *3*, 397–403.

(34) Halgren, T. A. Merck molecular-force field. 1. basis, form, scope, parametrization, and performance of mmff94. *J. Comput. Chem.* **1996**, *17*, 490–519.

(35) Halgren, T. A. Merck molecular-force field. 2. mmff94 van der Waals and electrostatic parameters for intermolecular interactions. *J. Comput. Chem.* **1996**, *17*, 520–552.

(36) Halgren, T. A. Merck molecular-force field. 3. molecular geometries and vibrational frequencies for mmff94. *J. Comput. Chem.* **1996**, *17*, 553–586.

(37) Halgren, T. A.; Nachbar, R. B. Merck molecular-force field. 4. conformational energies and geometries for mmff94. *J. Comput. Chem.* **1996**, *17*, 587–615.

(38) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T. and Still, W. C. MacroModel-An Integrated Software System for Modeling Organic and Bioorganic Molecules using Molecular Mechanics. *J. Comput. Chem.* **1990**, *11*, 440–467.

(39) Schrödinger, Inc., 1500 S. W. First Avenue, Suite 1180, Portland, OR 97201-5815.

(40) Grunberger, G.; Weston, W. M.; Patwardhan, R.; Rappaport, E. B. Rosiglitazone Once or Twice Daily Improves Glycemic Control in Patients with Type 2 Diabetes (T2D). *Diabetes* **1999**, *48* (Suppl. 1), A102.

(41) Krause, B. R.; Princen, H. M. G.; Lack of predictability of classical animal models for hypolididemic activity: a good time for the mice? *Atherosclerosis* **1998**, *140*, 15–24.

(42) Polivka, Z.; Holubek, J.; Svatek, E.; Dlabac, A.; Pucek, D.; Sedivy, Z.; Protiva, M. 3-(4-methylpiperazino)dibenzo[*b,f*]-1,2,4-trazolo-[4,3-*d*]-1,4-thiazepine and its 6-chloro and 12-chloro derivatives; synthesis and pharmacology. *Coll. Czech. Chem. Commun.* **1983**, *48*, 1465–1476.

(43) Goldberg, A. A.; Wragg, A. H. Spasmolytics Derived from Xanthen. *J. Chem. Soc.* **1957**, 4823–4829.

(44) Kuhn, R.; Breyer, U. Alkylation of benzene on a Al_2O_3-column. *Ann. Chem.* **1963**, *661*, 173–180.

(45) Perumattam, J.; Shao, C.; Confer, W. L. Studies on the Alkylation and Chlorination of Fluorenes: Preparation of 9-(2-hydroxyethyl)fluorene and 2,7-Dichloro-9-(2-hydroxyethyl)fluorene. *Synthesis* **1994**, *11*, 1181–1184.

(46) Hoffsommer, R. D.; Taub, D.; Wendler, N. L. The Homoallylic Rearangement in the Synthesis of Amitriptyline and Related Systems. *J. Org. Chem.* **1962**, *27*, 4134–4137.

(47) Hoffsommer, R. D.; Taub, D.; Wendler, N. L. Synthesis of a Cyclopropyl carbinol in the Amitripyline Series. *J. Med. Chem.* **1964**, *7*, 392–393.

(48) Sadowski, I.; Bell, B.; Broad, P.; Hollis, M. *GAL4* fusion vectors for expression in yeast and mammalian cells. *Gene* **1992**, *118*, 137–141.

(49) Webster, N.; Jin, J. R.; Green, S.; Hollis, M.; Chambon, P. The Yeast UASG Is a Transcriptional Enhancer In Human HeLa Cells In the Presence of the GAL4 trans-activator. *Cell* **1988**, *52*, 169–178.

(50) Ebdrup, S. et al. manuscript in progress.

(51) Cerenius, Y.; Ståhl, K.; Svensson, L. A.; Ursby, T.; Oskarsson, Å.; Albertsson, J.; Liljas, A. The crystallography beamline I711 at MAX II. *J. Synchrotron Radiat.* **2000**, *7*, 203–208.

(52) Kabsch, W. Automatic processing of rotation diffraction data from crystals of initially unknown symmetry and cell constants. *J. Appl. Crystallogr.* **1993**, *26*, 795–800.

(53) Brunger, A. T.; Krukowski, A.; Erickson, J. W. Slow-cooling protocols for crystallographic refinement by simulated annealing. *Acta Crystallogr.* **1990**, *A46*, 585–593.

(54) Oldfield, T. J.; Hubbard, R. E. Analysis of c-alpha geometry in protein structures. *Proteins* **1994**, *18*, 324–337.

(55) Kleywegt, G. J.; Jones, T. A. Databases in protein Crystallography. *Acta Crystallogr.* **1998**, *D54*, 1119–1131.

(56) Goodford, P. J. A computational procedure for determining energetically favorable binding sites on biologically important macromolecules *J. Med. Chem.* **1985**, *28*, 849–857.

(57) Boobbyer, D. N.; Goodford, P. J.; McWhinnie, P. M.; Wade, R. C. New hydrogen-bond potentials for use in determining energetically favorable binding sites on molecules of known structure. *J. Med. Chem.* **1989**, *32*, 1083–1094.

(58) Wade, R. C.; Goodford, P. J. Further development of hydrogen bond functions for use in determining energetically favorable binding sites on molecules of known structure. 2. Ligand probe groups with the ability to form more than two hydrogen bonds. *J. Med. Chem.* **1993**, *36*, 148–156.

(59) Molecular Discovery Ltd., 20a Berkeley Street, Mayfair, London W1X 5AE, U.K.

(60) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. Semianalytical Treatment of Solvation for Molecular Mechanics and Dynamics. *J. Am. Chem. Soc.* **1990**, *112*, 6127–6129.

(61) Goodman, J. M.; Still, W. C. An Unbounded Systematic Search of Conformational Space *J. Comput. Chem.* **1991**, *12*, 1110–1117.

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United States Patent [19]

Meli et al.

[11] Patent Number: 5,011,833

[45] Date of Patent: Apr. 30, 1991

[54] NOVEL DERIVATIVES OF 6,11-DIHYDRO-DIBENZO(C,F)(1,2,5)-THIADIAZEPINE 5,5-DIOXIDE, SALTS THEREOF AND APPROPRIATE PROCESSES FOR THE PREPARATION THEREOF

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[51] Int. Cl.[5] C07D 285/36; A61K 31/55

[52] U.S. Cl. 511/211; 540/545

[58] Field of Search 540/545; 514/211

[56] References Cited

U.S. PATENT DOCUMENTS

3,254,631	6/1966	Yale	540/550
3,268,557	8/1966	Weber	540/545
4,659,707	4/1987	Giano	540/495

FOREIGN PATENT DOCUMENTS

3235795	3/1984	Fed. Rep. of Germany	540/495

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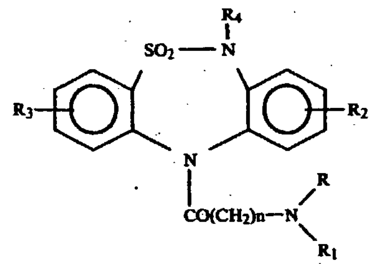
[57] ABSTRACT

Novel derivatives of 11-carbonyl-6,11-dihydrodibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide of the general formula



wherein

R and R_1, which can be different, represent a hydrogen atom or a (C_1–C_5) alkyl or (C_1–C_4)hydroxyalkyl group, or R and R_1 together can form a 5-membered and/or 6-membered heterocyclic ring which may contain a further heteroatom,

R_2 and R_3, which can be different, represent a hydrogen atom, a (C_1–C_3)alkoxy, (C_1–C_3)alkyl, nitro, amino or (C_1–C_3)alkylamino, halogen, halogeno-alkyl or hydroxyl group,

R_4 represents a hydrogen atom or a (C_1–C_4) alkyl, alkylaryl or (C_1–C_6)alkylamino group, and

n assumes values of 0, 1 or 2, and

non-toxic, pharmaceutically acceptable salts thereof, obtained by addition of acids or alkyl halides.

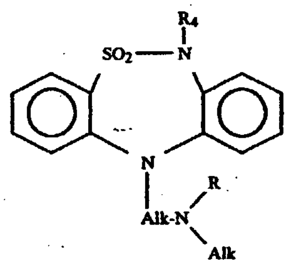
9 Claims, No Drawings

NOVEL DERIVATIVES OF 6,11-DIHYDRO-DIBENZO(C,F)(1,2,5)-THIADIAZEPINE 5,5-DIOXIDE, SALTS THEREOF AND APPROPRIATE PROCESSES FOR THE PREPARATION THEREOF

DESCRIPTION

The invention relates to novel derivatives of 6,11-dihydro-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide, to salts thereof, to pharmaceutical preparations containing these products and to methods for producing and using these derivatives.

Derivatives of 6,11-dihydro-dibenzo[c,f][1,2,5]-thiadiazepine 5,5-dioxide are already known in the literature. More in detail, U.S. Pat. Nos. 3,222,789 and 3,274,058 describe monoalkylaminoalkyl and dialkylaminoalkyl derivatives of 6,11-dihydro-dibenzo[c,f][1,2,5]-thiadiazepine 5,5-dioxide, corresponding to the structure:



where R can be hydrogen or another alkyl group.

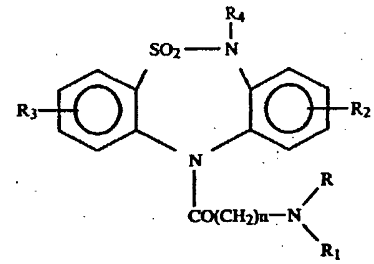
According to the two U.S. patents, the abovementioned compounds should be useful as psychotropic agents and as muscle relaxation agents. It is, however, known that toxicity studies have shown that such compounds can, even in sub-toxic doses, induce unpleasant side effects upon the cardiovascular system in experimental animals (A. Weber, J. Frossard, Ann. Pharm. Franc. 24, 1966, No. 6, 445–450). Such secondary effects therefore constitute an important limitation to the use of an antidepressant drug which normally must be administered over long periods of time.

It has now been found that the introduction of a carbonyl group

$$(>C=O),$$

to separate the nitrogen atom in the 11-position from the alkylaminoalkyl chain in the structure of 6,11-dihydro-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide, leads to a clear reduction in the side effects typical of this structure.

The present invention therefore relates to novel 11-carbonyl derivatives of dibenzothiadiazepine, of the general formula I:



I

wherein

R and R_1, which can be different, represent a hydrogen atom or a (C_1-C_5)alkyl or (C_1-C_4)-hydroxyalkyl group, or R and R_1 together can form a 5-membered and 6-membered heterocyclic ring which may have a further heteroatom,

R_2 and R_3, which can be different, represent a hydrogen atom, a (C_1-C_3)alkoxy, (C_1-C_3)alkyl, nitro, amino or (C_1-C_3)alkylamino, halogen, halogenoalkyl or hydroxyl group,

R_4 represents a hydrogen atom or a (C_1-C_4)alkyl, alkylaryl or (C_1-C_6)alkylamino group, and n assumes values of 0, 1 or 2.

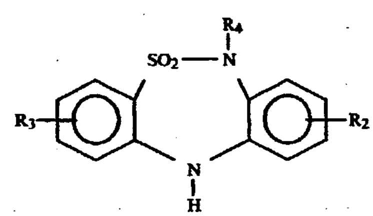
The compounds constituting the subject of the present patent application exhibit a psychotropic action equal to, and in some cases even greater than, that possessed by the compounds described in U.S. Pat. Nos. 3,274,058 and 3,322,789; in comparison with these latter, they have, however, the advantage of a lower incidence of side effects, and in particular are free of preconvulsive effects and do not alter the blood pressure.

The structural characteristic, which distinguishes the compounds of the present invention from analogs already known, resides in the presence of a keto group which separates the nitrogen atom in the 11-position from the alkyl substituent; in this way, the basic nitrogen becomes an amide nitrogen.

Compounds of this invention of particular interest are those in which R_2 and R_3 are hydrogen, R_4 is methyl, n assumes values of 1 or 2, and R and R_1 are hydrogen, methyl or ethyl.

The preferred compound of this invention is 6-methyl-6,11-dihydro-11-(N,N-dimethylaminoacetyl)-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.

The starting materials used for the preparation of the derivatives of the general formula I are, according to the method of the present invention, the corresponding 6,11-dihydro-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxides of the general formula II

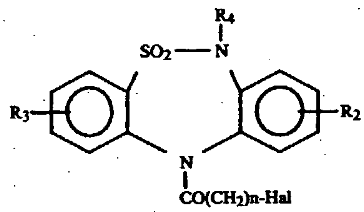


II

where the substituents R_2, R_3 and R_4 assume the meanings already defined for the compounds of the general formula I.

The method of the present invention comprises treating the compounds of the general formula II with the

appropriate ω-halogenoacyloyl halide to obtain the corresponding 6,11-dihydro-11-(ω-halogenaoalkyl)-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxides of the general formula III

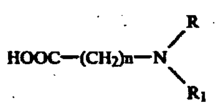


III

where R_2, R_3 and R_4 have the meaning already defined for the compounds of the general formula I, Hal stands for halogen and n assumes values of 1 or 2. The reaction is carried out in an organic solvent or in an excess of halogenoacyloyl halide, and with heating.

The compounds of the general formula III are not always purified, since they are sometimes used in the crude state. By treatment with the appropriate amine, or with ammonia or heterocyclic compound, such compounds either in the pure or crude form give the corresponding compounds of the general formula I; this reaction is preferably carried out in a polar solvent such as, for example, acetone, dioxane or alcohols at ambient temperature or under reflux.

Alternatively, the compounds of the general formula I, in which n=1 or 2, can be obtained by reacting 6,11-dihydro-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide with a suitable acid in an activated form such as, for example, an active ester or anhydride or chlorides, of the general formula:



where R and R_1 have the ready meaning al defined for the compounds of the general formula I and n assumes values of 1 and 2. Such a reaction is preferably carried out in an organic solvent, or in an excess of the acid, with heating.

The compounds of the general formula I, if n=0, are obtained by treating 6,11-dihydro-dibenzo[c,f][1,2,5]-thiadiazepine 5,5-dioxide with a derivative of carbonic acid such as, for example, trichloromethyl chloroformate or carbonyl chloride, and subsequent reaction of the intermediate obtained with ammonia or a substituted amine; the reaction is preferably carried out by treating the sodium salt of the compound of the general formula II.

EXAMPLE 1

Preparation of 6-6,11-dihydro-11-chloroacetyldibenzo[c,f][1,2,5]-thiadiazepine 5,5-dioxide

15 g (0.057 mol) of 6-methyl-6,11-dihydrodibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide in 100 ml of chloroacetyl chloride are kept for 2 hours under reflux. The mixture is poured into water and ice with stirring, and the oil which separates out solidifies. The solid is filtered off, washed with water and dried.

Crystallized from ethyl acetate: melting point 141°-3° C., quantity obtained 15.3 g, yield 80%.

In an analogous manner, other intermediates the general formula III are prepared, in particular the following:

6-Methyl-6,11-dihydro-11-(3-chloropropionyl)dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide, melting point 131°-132° C.

2-Chloro-3,6-dimethyl 6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide, melting point 164°-6° C. 6-Ethyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide, melting point 174°-176° C.

2-Chloro-6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide, melting point 183°-184° C.

6-Methyl-2-methoxy-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide, melting point 178°-9° C.

6-Propyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide, melting point 147°-48° C.

2-Trifluoromethyl-6-methyl-6,11-dihydro-11-chloroacetyldibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide, melting point 121°-122° C.

9-Chloro-6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 202°-203° C.

6,9-Dimethyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 155°-157° C.

8-Chloro-6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 158°-159° C.

3-Chloro-6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 209°-210° C.

EXAMPLE 2

6-Methyl-6,11-dihydro-11-[3-(N,N-dimethylamino)propionyl]dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide hydrochloride

5.5 g (0.037 mol) of sodium iodide are added to 13 g (0.037 mol) of 6-methyl-6,11-dihydro-11-(3-chloropropionyl)-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide in 100 ml of acetone, and the mixture is heated under reflux for 30 minutes. After cooling, 7 ml of aqueous 33% dimethylamine are added, and the mixture is kept with stirring at ambient temperature for 16 hours. It is concentrated and the residue is treated with 5% HCl, the insoluble fraction is filtered off and the filtrate is alkalized with $NaHCO_3$; a solid forms, which is filtered off, washed with water and dried.

Crystallized from 95° ethanol, melting point 189°-90° C., quantity obtained 10 g, yield 68%.

EXAMPLE 3

6-Methyl-6,11-dihydro-11-carbamoyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide

0.9 g (0.027 mol) of 80% sodium hydride are added to 7 g (0.027 mol) of 6-methyl-6,11-dihydro-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide in 200 ml of dioxane, and the mixture is heated for 30 minutes at 80° C. After cooling, 5.4 g of trichloromethyl chloroformate are added dropwise, with cooling in an ice bath, and the mixture is left to stand overnight. Gaseous NH_3 is then introduced up to saturation, the mixture is filtered, and

the filtrate is concentrated and diluted with water. This gives a solid which is filtered, washed and dried.

Crystallized from ethanol, melting point 240°-2° C., quantity obtained 4.9 g, yield 60%.

EXAMPLE 4

Proceeding as described for the two preceding compounds, the following further compounds of the general formula I are prepared, which are reported here with their respective melting points:

1. 6-Methyl-6,11-dihydro-11-[(4-methyl-piperazin-1-yl)acetyl]-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 143°-44° C., obtained according to Example 2 from 6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.
2. 6-Methyl-6,11-dihydro-11-{[4-(2-hydroxyethyl)piperazin-1-yl]-acetyl}-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide dihydrochloride: melting point 236°-38° C., obtained according to Example 2 from 6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]-thiadiazepine 5,5-dioxide.
3. 6-Methyl-6,11-dihydro-11-(N,N-dimethylaminoacetyl)dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 179°-181° C., hydrochloride n H_2O: m.p. 198° C. dec. (isopropanol/H_2O), obtained according to Example 2 from 6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.
4. 2-Chloro-3,6-dimethyl-6,11-dihydro-11-[(4-methyl-piperazin-1-yl)-acetyl]-dibenzo [c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 156°-58° C., obtained according to Example 2 from 2-chloro-3,6-dimethyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]-thiadiazepine 5,5-dioxide.
5. 2-Chloro-3,6-dimethyl-6,11-dihydro-11-[(4-phenyl-piperazin-1-yl)-acetyl]-dibenzo [c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 194°-96° C. obtained according to Example 2 from 2-chloro-3,6-dimethyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]-thiadiazepine 5,5-dioxide.
6. 2-Chloro-3,6-dimethyl-6,11-dihydro-11-{[4-(2-hydroxyethyl)-piperazin-1-yl]-acetyl}-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide dihydrochloride: melting point 234°-36° C., obtained according to Example 2 from 2-chloro-3,6-dimethyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.
7. 2-Chloro-3,6-dimethyl-6,11-dihydro-11-{[4-(pyrimidin-2-yl)-piperazin-1-yl]-acetyl}-dibenzo[c,f][1,2,5]-thiadiazepine 5,5-dioxide: melting point 180°-82° C., obtained according to Example 2 from 2-chloro-3,6-dimethyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.
8. 2-Chloro-3,6-dimethyl-6,11-dihydro-11-(N-isopropylamino-acetyl)-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide hydrochloride: melting point 248°-50° C., obtained according to Example 2 from 2-chloro-3,6-dimethyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.
9. 2-Chloro-3,6-dimethyl-6,11-dihydro-11-(N,N-dimethylamino-acetyl)-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide hydrochloride: melting point 240°-42° C., obtained according to Example 2 from 2-chloro-3,6-dimethyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.
10. 2-Methoxy-6-methyl-6,11-dihydro-11-[N,N-dimethylamino-acetyl)-dibenzo[c,f][1,2,5]thiadiazepine 5,5dioxide hydrochloride: melting point 270°-72°, according to Example 2 from 2-methoxy-6obtained methyl-6,11-dihdyro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.
11. 6-Methyl-6,11-dihdyro-11-(N,N-dimethylcarbonyl)-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 170°-71° C., obtained according to Example 3 from 6-methyl-6,11-dihydro-dibenzo[c,f][1,2,5]-thiadiazepine 5,5-dioxide.
12. 6-Ethyl-6,11-dihydro-11-(N,N-dimethylamino-acetyl)dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 115°-116° C., obtained according to Example 2 from 6-ethyl-6,11-dihydro-11-chloroacetyldibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.
13. 6-Methyl-6,11-dihydro-11-(N,N-diethylamino-acetyl)dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 161°-62° C., obtained according to Example 2 from 6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.
14. 6-Methyl-6,11-dihydro-11-(N-isopropylamino-acetyl)dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 115°-17° C., obtained according to Example 2 from 6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.
15. 6-Methyl-6,11-dihydro-11-(N-t-butylamino-acetyl)-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 143°-45° C., obtained according to Example 2 from 6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.
16. 6-Methyl-6,11-dihydro-11-[(morpholin-1-yl)-acetyl]dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 129°-31° C., obtained according to Example 2 from 6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.
17. 6-Methyl-6,11-dihydro-11-[(4-methylpiperidin-1-yl)acetyl]-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 159°-60° C., obtained according to Example 2 from 6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.
18. 6-Methyl-6,11-dihydro-11-[(pyrrolidin-1-yl)-acetyl]-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 175°-77° C., obtained according to Example 2 from 6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.
19. 6-Methyl-6,11-dihydro-11- (N-methylamino-acetyl)-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 135°-37° C., obtained according to Example 2 from 6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.
20. 2-Chloro-6-methyl-6,11-dihydro-11-(N,N-dimethylamino-acetyl)-dibenzo[c,f][1,2,5]thiadiazepine 5,5dioxide: melting point 138°-39° C., obtained according to Example 2 from 2-chloro-6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5dioxide.
21. 6-Propyl-6,11-dihydro-11-(N,N-dimethylamino-acetyl)dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide hydrochloride: melting point 147°-149° C., obtained according to Example 2 from 6-propyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5dioxide.
22. 9-Chloro-6-methyl-6,11-dihydro-11-(N,N-dimethylamino-acetyl)-dibenzo[c,f][1,2,5]thiadiazepine 5,5dioxide: melting point 175°-176° C., obtained according to Example 2 from 9-chloro-6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.

23. 6,9-dimethyl-6,11-dihydro-11-(N,N-dimethylaminoacetyl)-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 135°–137° C., obtained according to Example 2 from 6,9-dimethyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5thiadiazepine 5,5-dioxide.

24. 8-chloro-6-methyl-6,11-dihydro-11-(N,N-dimethylamino-acethyl)-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 132°–133° C., obtained according to Example 2 from 8-chloro-6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.

25. 3-chloro-6-methyl-6,11-dihydro-11-(N,N-dimethylaminoacetil)dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide hydrochloride: melting point 163°–165° C. dec., obtained according to Example 2 from 3-chloro-6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.

26. 2-trifluoromethyl-6-methyl-6,11-dihydro-11-(N,N-dimethylamino-acetyl)dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide: melting point 156°–157° C., obtained according to Example 2 from 2-trifluoromethyl-6-methyl-6,11-dihydro-11-chloroacetyl-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.

The pharmacological evaluation was carried out by means of various tests which predict the psychotropic activity, in particular that of the antidepressant type (Willner, 1984, Psychopharmacology 83:1). The compounds of the present patent are active, for example in the "apomorphine test" (Puech et al., 1981, Psychopharmacology 75:84). In particular, 6-methyl-6,11-dihydro-11- N,N-dimethylamino-acetyl)dibenzo [c,f][1,2,5]thiadiazepine 5,5-dioxide has the following pharmaceutical characteristics: (a) ED-50 in counteracting the hypothermia induced by apomorphine (Puech et al., 1981, Psychopharmacology 75:84) in mice=13.5 mg/kg by the oral route and 4.7 mg/kg by the intraperitoneal route; (b) minimum oral dose effective in counteracting reserpine hypothermia (Bourin et al., 1983, Arzneim-Forsch/Drug Res 33:1173) in mice=25 mg/kg; (c) oral LD-50 in mice 1920 mg/kg; (d) does not induce convulsions; (e) does not alter the motor activity; (f) does not alter the blood pressure; (g) induces very weak anticholinergic effects; (h) is not cardiotoxic; (i) reduces duodenal ulcers.

Table 1 summarizes the test results.

Under the same experimental conditions used by us, 6-methyl-11-(3-dimethylaminopropyl)-6,11-dihydrodibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide (one of the most interesting compounds from U.S. Pat. No. 3,274,058) shows the following pharmacological characteristics: (a) minimum oral dose effective in counteracting reserpine hypothermia in mice=100 mg/kg; (b) oral LD-50 in mice=655 mg/kg; (c) induces convulsions; (d) increases the motor activity; (e) increases the blood pressure; (f) possesses anticholinergic effect.

The compounds to which the present application refers are suitable for either oral or parenteral or suppositorial administration in the form of: tablets, capsules, powders, granules, syrups, gel spray, lotions, suspensions, injectable solutions and suppositories.

The pharmaceutical formulations suitable for administration contain the compounds, which are the subject of the invention, in a quantity of between 0.1 and 30%, preferably between 0.5 and 10% by weight, in a mixture with the usual excipients such as, for example: gelling agents, suppository bases, auxiliaries for tablets and other excipients for the active ingredients such as, for example, antioxidants, dispersants, emulsifiers, antifoams, flavor improvers, preservatives, solubilizers and colorants.

It is advisable to administer the active compound in one or more daily doses of between 0.1 and 50 mg/kg body weight, preferably between 0.5 and 20 mg/kg body weight.

The optimum doses and the administration route of the active compounds, required in any particular case, are easily determined by any person skilled in the art in accordance with his experience.

The present invention also comprises pharmaceutical formulations which represent combinations of one or more compounds, which are the subject of the invention, as pure formulations representing combinations of one or more compounds, subject of the invention, and one or more compounds which are pharmaceutically active towards the central nervous system.

TABLE 1

Compound	Hypothermia inhibition (*) from Apo16: ED-50	LD-50 (mg/kg/os) (**)
1	++	1500–2000
2	++	750–1000
3	+	greater than 2000
4	+	500–700
5	+	1000–1500
6	++	1500–2000
imipramine	++	less than 350

(*) Apomorphine 16 mg/kg (Apo16) was administered to Swiss male mice by the subcutaneous route 30 minutes before measuring the rectal temperature. The compounds were administered by the oral route 60 minutes before measuring the rectal temperature.

ED-50 = dose of compound which reduces by 50% the hypothermia caused by Apomorphine administered at 16 mg/kg by the subcutaneous route:

++ = ED-50 comprised in the range of 4–15 mg/kg/os

+ = ED-50 comprised in the range of 15–25 mg/kg/os

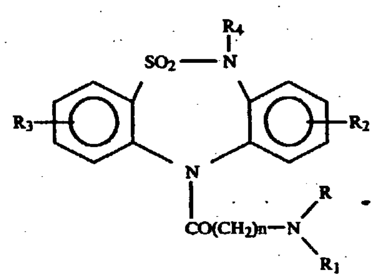
(**) LD-50 = dose of compound which causes the death of 50% of the mice (Swiss male mice) after 14 days.

Compounds:

1. 6-methyl-6,11-dihydro-11-(N,N-dimethylamino-acetyl)dibenzo c,f 1,2,5 thiadiazepine 5,5-dioxide
2. 6-methyl-6,11-dihydro-11-(N-methylamino-acetyl)dibenzo c,f 1,2,5 thiadiazepine 5,5-dioxide
3. 6-methyl-6,11-dihydro-11-(N,N-diethylamino-acetyl)dibenzo c,f 1,2,5 thiadiazepine 5,5-dioxide
4. 6-methyl-6,11-dihydro-11-3-(N,N-dimethylamino)propionyl dibenzo c,f 1,2,5 thiadiazepine 5,5-dioxide
5. 8-chloro-6-methyl-6,11-dihydro-11-(N,N-dimethylamino-acetyl)dibenzo c,f 1,2,5 thiadiazepine 5,5-dioxide
6. 6,9-dimethyl-6,11-dihydro-11-(N,N-dimethylamino-acetyl)dibenzo c,f 1,2,5 thiadiazepine 5,5-dioxide

We claim:

1. A novel derivative of 11-carbonyl-6,11-dihydrodibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide of the general formula



wherein

R and R_1, which can be different, represent a hydrogen atom or a (C_1–C_5)alkyl or (C_1–C_4)hydroxyalkyl group, or R and R_1 together can form a 5-membered and/or 6-membered heterocyclic ring which may contain a further heteroatom,

R_2 and R_3, which can be different, represent a hydrogen atom, a (C_1-C_3)alkoxy, (C_1-C_3)alkyl, nitro, amino or (C_1-C_3)alkylamino, halogen, halogenoalkyl or hydroxyl group,

R_4 represents a hydrogen atom or a (C_1-C_4)alkyl, alkylaryl or (C_1-C_6)alkylamino group, and n assumes values of 0, 1 or 2.

2. A non-toxic, pharmaceutically acceptable salt of a compound as claimed in claim 1, obtained by addition of an acid or alkyl halide.

3. A compound as claimed in claim 1, wherein R_2 and R_3 are hydrogen, R_4 is methyl, n is 1 or 2, and R and R_1 are hydrogen, methyl or ethyl.

4. A compound as claimed in claim 3, selected from the group comprising:

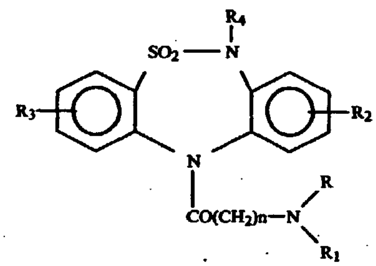
6-methyl-6,11-dihydro-11-(N,N-dimethylamino-acetyl)dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide,
6-methyl-6,11-dihydro-11-[3-(N,N-dimethylamino)-propionyl]-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide,
6-methyl-6,11-dihydro-11-(N,N-diethylamino-acetyl)dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide,
6-methyl-6,11-dihydro-11-(N-methylamino-acetyl)-dibenzo[c,f][1,2,5-thiadiazepine 5,5-dioxide,
6,9-dimethyl-6,11-dihydro-11-(N,N-dimethylamino-acetyl)dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide,
8-chloro-6-methyl-6,11-dihydro-11-(N,N-dimethyl-amino-acetyl)dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.

5. A pharmaceutically acceptable acid addition salt of 6-methyl-6,11-dihydro-11-(N,N-dimethylamino-acetyl)-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide.

6. Pharmaceutical composition comprising an active dose for action on the central nervous system for antidepressant action, of at least one compound of claim 1 together with a pharmaceutically acceptable vehicle.

7. Composition of claim 6 in a pharmaceutical form suitable for oral, parenteral or rectal administration.

8. Pharmaceutical composition comprising an effective quantity of a compound acting on the central nervous system by its antidepressant action, selected from the group comprising derivatives of 11-carbonyl-6,11-dihydro-dibenzo[c,f][1,2,5]thiadiazepine 5,5-dioxide of the formula



where R, R_1, R_2, R_3, R_4 and n have the meaning given for them in claim 1.

9. A pharmaceutical composition comprising an active dose for its action on the central nervous system by antidepressant action, of a compound of claim 4 in a pharmaceutical form suitable for oral, parenteral or rectal administration.

* * * * *

Attachment A

ALLOWANCE HOT LIST

Appl. No. 9/995,137 Prepared by P. Stanback
Examiner-TC Truong Date 3/2/04

JACKET:

YES NO Primary Examiner box complete.
YES NO Issuing Classification complete.

PTO-892/1449:

YES NO Examiner's initials or cross-through lines supplied for each item cited by applicant.
YES NO Date(s) supplied/complete on all PTO-1449/892 sheets. (Month and year required.)

SPEC:
YES NO Brief Description of Drawings includes description of each figure in drawings.
YES NO Continuing data is mentioned in 1st paragraph. (Can be an insert.)

CLAIMS:

YES NO Claims listed on Notice of Allowability match allowed claims and/or index of claims.
YES NO Claims correctly numbered in index.
(No duplicate or missing claim numbers.)
(No incorrect dependencies.)

CRFE:
YES NO If necessary (biological sequence listing).

NOTICE OF ALLOWABILITY:

YES NO Either Box No. 3 (drawings accepted) or Box No. 8 (corrected drawing request) has been checked.